into the periplasm. The secretion of 2 native E. coli proteins was unaffected by *TIR"** strength when tested over an identical range. (39 ref)

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26jul00 15:16:50 User219783 Session D1612.2

Devi, S.
09/189415



-key terms

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     FILE 'REGISTRY' ENTERED AT 15:13:02 ON 26 JUL 2000
                E INTIMIN/CN
L1            16 SEA ABB=ON  PLU=ON  INTIMIN ?/CN
L2             2 SEA ABB=ON  PLU=ON  TRANSLOCATED INTIMIN RECEPTOR?/CN

     FILE 'CAPLUS' ENTERED AT 15:14:21 ON 26 JUL 2000
L3           616 SEA ABB=ON  PLU=ON  L2 OR TIR OR TRANSLOCAT? INTIMIN OR
                EAEA OR EAE A
L4            38 SEA ABB=ON  PLU=ON  L3 AND ((CITROBACTER? OR CITRO
                BACTER?)(W)RODENT? OR (HAFNIA OR H)(W)ALVEI OR EPEC OT
                ETEC OR ENTERPATHOG? OR ENTEROHEMORRH? OR ENTEROHAEMORRH
                ? OR ENTERO(W)(PATHOG? OR HEMORRH? OR HAEMORRH?))(S)COLI)
L5            19 SEA ABB=ON  PLU=ON  L4 AND (L1 OR INTIMIN)
L6             1 SEA ABB=ON  PLU=ON  L4 AND (SDSPAGE OR SDS PAGE)
L7            19 SEA ABB=ON  PLU=ON  L5 OR L6
```

misspelling; see last 3 pgs.; L27→

L7 ANSWER 1 OF 19 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 2000:497087 CAPLUS

TITLE: Crystal structure of enteropathogenic Escherichia coli **intimin**-receptor complex

AUTHOR(S): Luo, Yu; Frey, Elizabeth A.; Pfuetzner, Richard A.; Creagh, A. Louise; Knoechel, Derek G.; Haynes, Charles A.; Inlay, B. Brett; Strynadka, Natalie C. J.

CORPORATE SOURCE: Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, BC, V6T 1Z3, Can.

SOURCE: Nature (London) (2000), 405(6790), 1073-1077
CODEN: NATUAS; ISSN: 0028-0836

PUBLISHER: Nature Publishing Group

DOCUMENT TYPE: Journal

LANGUAGE: English

AB **Intimin** and its **translocated intimin** receptor (**Tir**) are bacterial proteins that mediate adhesion between mammalian cells and attaching and effacing (A/E) pathogens. Enteropathogenic Escherichia coli (EPEC) causes significant paediatric morbidity and mortality world-wide. A related A/E pathogen, **enterohaemorrhagic** E. **coli** (EHEC; 0157:H7) is one of the most important food-borne pathogens in North America, Europe and Japan. A unique and essential feature of A/E bacterial pathogens is the formation of actin-rich pedestals beneath the intimately adherent bacteria and localized destruction of the intestinal brush border. The bacterial outer membrane adhesin, **intimin**, is necessary for the prodn. of the A/E lesion and diarrhea. The A/E bacteria translocate their own receptor for **intimin**, **Tir**, into the membrane of mammalian cells using the type III secretion system. The translocated **Tir** triggers addnl. host signalling events and actin nucleation, which are essential for lesion formation.

Searcher : Shears 308-4994

Here we describe the the crystal structures of an EPEC **intimin** carboxyterminal fragment alone and in complex with the EPEC **Tir** **intimin**-binding domain, giving insight into the mol. mechanisms of adhesion of A/E pathogens.

L7 ANSWER 2 OF 19 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 2000:472429 CAPLUS
TITLE: Structural basis for recognition of the **translocated intimin** receptor (**Tir**) by **intimin** from enteropathogenic Escherichia coli
AUTHOR(S): Batchelor, Miranda; Prasannan, Sunil; Daniell, Sarah; Reece, Stephen; Connerton, Ian; Bloomberg, Graham; Dougan, Gordon; Frankel, Gad; Matthews, Stephen
CORPORATE SOURCE: Department of Biochemistry, Imperial College of Science, Technology and Medicine, London, SW7 2AZ, UK
SOURCE: EMBO J. (2000), 19(11), 2452-2464
CODEN: EMJODG; ISSN: 0261-4189
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Intimin** is a bacterial adhesion mol. involved in intimate attachment of enteropathogenic and **enterohaemorrhagic** Escherichia **coli** to mammalian host cells. **Intimin** targets the **translocated intimin** receptor (**Tir**), which is exported by the bacteria and integrated into the host cell plasma membrane. In this study we localized the **Tir**-binding region of **intimin** to the C-terminal 190 amino acids (Int190). We have also detd. the region's high-resoln. soln. structure, which comprises an Ig domain that is intimately coupled to a novel C-type lectin domain. This fragment, which is necessary and sufficient for **Tir** interaction, defines a new super domain in **intimin** that exhibits striking structural similarity to the integrin-binding domain of the Yersinia invasin and C-type lectin families. The extracellular portion of **intimin** comprises an articulated rod of Ig domains extending from the bacterium surface, conveying a highly accessible "adhesive tip" to the target cell. The interpretation of NMR-titrn. and mutagenesis data has enabled us to identify, for the first time, the binding site for **Tir**, which is located at the extremity of the Int190 moiety.
REFERENCE COUNT: 59
REFERENCE(S): (1) Adu-Bobie, J; J Clin Microbiol 1998, V36, P662 CAPLUS
(2) Bax, A; Magn Res 1990, V88, P425 CAPLUS
(3) Boyington, J; Immunity 1999, V10, P75 CAPLUS
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CAPLUS
(6) de Grado, M; Cell Microbiol 1999, V1, P7
CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 3 OF 19 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 2000:11301 CAPLUS
DOCUMENT NUMBER: 132:176532
TITLE: Quorum sensing controls expression of the type III secretion gene transcription and protein secretion in **enterohemorrhagic** and enteropathogenic Escherichia **coli**
AUTHOR(S): Sperandio, Vanessa; Mellies, Jay L.; Nguyen, William; Shin, Sooan; Kaper, James B.
CORPORATE SOURCE: Center for Vaccine Development and Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD, 21201, USA
SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1999), 96(26), 15196-15201
CODEN: PNASA6; ISSN: 0027-8424
PUBLISHER: National Academy of Sciences
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Enterohemorrhagic** Escherichia **coli** O157:H7 and enteropathogenic E. **coli** cause a characteristic histopathol. in intestinal cells known as attaching and effacing. The attaching and effacing lesion is encoded by the Locus of Enterocyte Effacement (LEE) pathogenicity island, which encodes a type III secretion system, the **intimin** intestinal colonization factor, and the **translocated intimin** receptor protein that is translocated from the bacterium to the host epithelial cells. Using lacZ reporter gene fusions, the authors show that expression of the LEE operons encoding the type III secretion system, **translocated intimin** receptor, and **intimin** is regulated by quorum sensing in both **enterohemorrhagic** E. **coli** and enteropathogenic E. **coli**. The luxS gene recently shown to be responsible for prodn. of autoinducer in the Vibrio harveyi and E. coli quorum-sensing systems is responsible for regulation of the LEE operons, as shown by the mutation and complementation of the luxS gene. Regulation of intestinal colonization factors by quorum sensing could play an important role in the pathogenesis of disease caused by these organisms. These results suggest that intestinal colonization by E. coli O157:H7, which has an unusually low infectious dose, could be induced by quorum sensing of signals produced by nonpathogenic E. coli of the normal intestinal flora.
REFERENCE COUNT: 41
REFERENCE(S): (4) Baca-Delancey, R; Proc Natl Acad Sci USA

1999, V96, P4610 CAPLUS
(5) Donnenberg, M; Infect Immun 1991, V59, P4310 CAPLUS
(6) Donnenberg, M; J Bacteriol 1993, V175, P4670 CAPLUS
(7) Elliott, S; Mol Microbiol 1998, V28, P1 CAPLUS
(8) Elliott, S; Mol Microbiol 1999, V33, P1176 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 4 OF 19 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1999:675935 CAPLUS
DOCUMENT NUMBER: 132:20883
TITLE: The **Tir**-binding region of **enterohemorrhagic** Escherichia **coli intimin** is sufficient to trigger actin condensation after bacterial-induced host cell signalling
AUTHOR(S): Liu, Hui; Magoun, Loranne; Luperchio, Steve; Schauer, David B.; Leong, John M.
CORPORATE SOURCE: Department of Molecular Genetics and Microbiology, University of Massachusetts Medical Center, Worcester, MA, 01655, USA
SOURCE: Mol. Microbiol. (1999), 34(1), 67-81
CODEN: MOMIEE; ISSN: 0950-382X
PUBLISHER: Blackwell Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Enterohemorrhagic** Escherichia **coli** (EHEC) has emerged as an important agent of diarrheal disease. Attachment to host cells, an essential step during intestinal colonization by EHEC, is assocd. with the formation of a highly organized cytoskeletal structure contg. filamentous actin, termed an attaching and effacing (A/E) lesion, directly beneath bound bacteria. The outer membrane protein **intimin** is required for the formation of this structure, as is **Tir**, a bacterial protein that is translocated into the host cell and is thought to function as a receptor for **intimin**. To understand **intimin** function better, the authors fused EHEC **intimin** to a homologous protein, Yersinia pseudotuberculosis invasin, or to maltose-binding protein. The N-terminal 539 amino acids of **intimin** were sufficient to promote outer membrane localization of the C-terminus of invasin and, conversely, the N-terminal 489 amino acids of invasin were sufficient to promote the localization of the C-terminus of **intimin**. The C-terminal 181 residues of **intimin** were sufficient to bind mammalian cells that had been preinfected with an enteropathogenic E. coli strain that expresses **Tir** but not **intimin**.

Searcher : Shears 308-4994

Binding of **intimin** derivs. to preinfected cells correlated with binding to recombinant **Tir** protein. Finally, the 181-residue minimal **Tir**-binding region of **intimin**, when purified and immobilized on latex beads, was sufficient to trigger A/E lesions on preinfected mammalian cells.

REFERENCE COUNT: 60

REFERENCE(S):
(3) Deibel, C; Mol Microbiol 1998, V28, P463 CAPLUS
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ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 5 OF 19 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1999:529342 CAPLUS
DOCUMENT NUMBER: 131:155518
TITLE: Methods, kits and vaccines for detecting, typing, treating and isolating **intimin**-expressing microorganisms
INVENTOR(S): Batchelor, Miranda; Dougan, Gordon; Frankel, Gad
PATENT ASSIGNEE(S): Imperial College of Science, Technology and Medicine, UK
SOURCE: PCT Int. Appl., 57 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 9941614 | A2 | 19990819 | WO 1999-GB467 | 19990216 |
| WO 9941614 | A3 | 19991007 | | |

W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

| | | | | |
|---|---|---|---|---|
| AU 9925356 | A1 | 19990830 | AU 1999-25356 | 19990216 |
| PRIORITY APPLN. INFO.: | | | GB 1998-3322 | 19980216 |
| | | | WO 1999-GB467 | 19990216 |

Searcher : Shears 308-4994

AB Methods of detecting and/or diagnosing **intimin**-expressing microorganisms such as enteropathogenic Escherichia **coli** (EPEC), **enterohemorrhagic** E. **coli**, **Citrobacter rodentium**, and/or RDEC-1 (rabbit diarrheagenic E. **coli** strain), as well as kits for use in such methods are provided. In particular, methods based antisera raised against conserved regions are described, as well as polypeptide regions useful in the prodn. of antisera. Vaccines based on such peptides are also described herein. In addn., methods of typing/classification of such bacteria are also described herein. Finally, methods for the isolation of **intimin**-expressing microorganisms are also provided. Using a combination of antisera and PCR it was possible to distinguish between 5 different **intimin** types.

L7 ANSWER 6 OF 19 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1999:500094 CAPLUS
DOCUMENT NUMBER: 131:255689
TITLE: Role of bacterial **intimin** in colonic hyperplasia and inflammation
AUTHOR(S): Higgins, Lisa M.; Frankel, Gad; Connerton, Lan; Goncalves, Nathalie S.; Dougan, Gordon; MacDonald, Thomas T.
CORPORATE SOURCE: Department of Paediatric Gastroenterology, St. Bartholomews and the Royal London School of Medicine and Dentistry, London, EC1A 7BE, UK
SOURCE: Science (Washington, D. C.) (1999), 285(5427), 588-591
CODEN: SCIEAS; ISSN: 0036-8075
PUBLISHER: American Association for the Advancement of Science
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Enteropathogenic Escherichia coil (EPEC) cells adhere to gut epithelial cells through **intimin** a: the Ligand for a bacterially derived epithelial transmembrane protein called the **translocated intimin** receptor. **Citrobacter rodentium** colonizes the mouse colon in a similar fashion and uses a different **intimin**: **intimin** .beta.. **Intimin** .alpha. was found to costimulate submitogenic signals through the T cell receptor. Dead **intimin** .beta.+ C. rodentium, **intimin** .alpha.-transfected C. rodentium or E. coli strain K12, and EPEC induced mucosal hyperplasia identical to that caused by C. rodentium live infection, as well as a massive T helper cell-type 1 immune response in the colonic mucosa. Mutation of cysteine-937 of **intimin** to alanine reduced costimulatory activity in vitro and prevented immunopathol. in vivo. The mucosal changes elicited by C. rodentium were interferon-.gamma.-dependent. Immunopathol.

induced by **intimin** enables the bacteria to promote conditions that are favorable for increased microbial colonization.

REFERENCE COUNT: 24

REFERENCE(S):
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(7) Frankel, G; Infect Immun 1994, V62, P1835 CAPLUS
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ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 7 OF 19 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1999:326051 CAPLUS
DOCUMENT NUMBER: 130:333761
TITLE: Pathogenic Escherichia coli **intimin** receptor **Tir** and gene **tir** and methods for detecting gene **tir** or **Tir** protein and for drug screening
INVENTOR(S): Finlay, B. Brett; Kenny, Brendan; Devinney, Rebekah; Stein, Marcus
PATENT ASSIGNEE(S): University of British Columbia, Can.
SOURCE: PCT Int. Appl., 91 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 9924576 | A1 | 19990520 | WO 1998-CA1042 | 19981110 |
| W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | | |
| AU 9911373 | A1 | 19990531 | AU 1999-11373 | 19981110 |
| PRIORITY APPLN. INFO.: | | | US 1997-65130 | 19971112 |
| | | | WO 1998-CA1042 | 19981110 |

AB A polypeptide, called **Tir** (for **translocated intimin** receptor), which is secreted by attaching and

effacing pathogens, such as the enteropathogenic (EPEC) and **enterohemorrhagic** (EHEC) E. **coli** is disclosed. These bacterial pathogens insert their own receptors into mammalian cell surfaces, to which the bacterial pathogen then adheres to trigger addnl. host signaling events and actin nucleation. Diagnosis of disease caused by pathogenic E. coli can be performed by the use of antibodies which bind to **Tir** to detect the protein or the use of nucleic acid probes for detection of nucleic acids encoding **Tir** polypeptide. Isolated nucleic acid sequences encoding **Tir** polypeptide, **Tir** peptides, a recombinant method for producing recombinant **Tir**, antibodies which bind to **Tir**, and a kit for the detection of **Tir**-producing E. coli are provided. A method of immunizing a host with **Tir** to induce a protective immune response to **Tir** or a second polypeptide of interest is also provided. A method for screening for compds. which interfere with the binding of bacterial pathogens to their receptors is further provided. Thus, protein Hp90, previously believed to be a host membrane protein, has been identified as an EHEC- or EPEC-secreted protein which acts as an **intimin** receptor. Proteins encoded by the espA and espB genes were necessary for delivery of **Tir** to the host membrane.

IT **200662-09-5P 224307-15-7P**
RL: ANT (Analyte); BOC (Biological occurrence); BPN (Biosynthetic preparation); BPR (Biological process); PRP (Properties); ANST (Analytical study); BIOL (Biological study); OCCU (Occurrence); PREP (Preparation); PROC (Process)
(amino acid sequence; pathogenic Escherichia coli **intimin** receptor **Tir** and gene **tir** and methods for detecting gene **tir** or **Tir** protein and for drug screening)

REFERENCE COUNT: 6
REFERENCE(S):
(1) Deibel, C; Molecular Microbiology 1998, V28(3), P463 CAPLUS
(2) Kenny, B; Cell 1997, V91, P511 CAPLUS
(3) Kenny, B; Infection and Immunity 1997, V65(7), P2528 CAPLUS
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(5) Paton, A; Infection and Immunity 1998, V66(11), P5580 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 8 OF 19 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1999:291203 CAPLUS
DOCUMENT NUMBER: 131:85390
TITLE: **Enterohemorrhagic** Escherichia **coli** O157:H7 produces **Tir**, which is translocated to the host cell membrane but is not tyrosine phosphorylated

AUTHOR(S): DeVinney, Rebekah; Stein, Markus; Reinscheid, Dieter; Abe, Akio; Ruschkowski, Sharon; Finlay, B. Brett
CORPORATE SOURCE: Biotechnology Laboratory, University of British Columbia, Vancouver, BC, V6T 1ZA, Can.
SOURCE: Infect. Immun. (1999), 67(5), 2389-2398
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Intimate attachment to the host cell leading to the formation of attaching and effacing (A/E) lesions is an essential feature of **enterohemorrhagic** Escherichia **coli** (EHEC) O157:H7 pathogenesis. In a related pathogen, enteropathogenic E. coli (EPEC), this activity is dependent upon translocation of the **intimin** receptor, **Tir**, which becomes tyrosine phosphorylated within the host cell membrane. In contrast, the accumulation of tyrosine-phosphorylated proteins beneath adherent EHEC bacteria does not occur, leading to questions about whether EHEC uses a **Tir**-based mechanism for adherence and A/E lesion formation. In this report, we demonstrate that EHEC produces a functional **Tir** that is inserted into host cell membranes, where it serves as an **intimin** receptor. However, unlike in EPEC, in EHEC **Tir** is not tyrosine phosphorylated yet plays a key role in both bacterial adherence to epithelial cells and pedestal formation. EHEC, but not EPEC, was unable to synthesize **Tir** in Luria-Bertani medium but was able to secrete **Tir** into M9 medium, suggesting that **Tir** synthesis and secretion may be regulated differently in these two pathogens. EHEC **Tir** and EPEC **Tir** both bind **intimin** and focus cytoskeletal rearrangements, indicating that tyrosine phosphorylation is not needed for pedestal formation. EHEC and EPEC **intimins** are functionally interchangeable, but EHEC **Tir** shows a much greater affinity for EHEC **intimin** than for EPEC **intimin**. These findings highlight some of the differences and similarities between EHEC and EPEC virulence mechanisms, which can be exploited to further define the mol. basis of pedestal formation.
REFERENCE COUNT: 45
REFERENCE(S):
(1) Abe, A; Infect Immun 1997, V65, P3547 CAPLUS
(4) Bieber, D; Science 1998, V280, P2114 CAPLUS
(5) Brunder, W; V Mol:Microbiol 1997, V24, P767 CAPLUS
(6) Dean-Nystrom, E; Infect Immun 1998, V66, P4560 CAPLUS
(7) Deibel, C; Mol Microbiol 1998, V28, P463 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 9 OF 19 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1999:159076 CAPLUS
DOCUMENT NUMBER: 130:308856
TITLE: Phosphorylation of tyrosine 474 of the enteropathogenic Escherichia coli (EPEC) **Tir** receptor molecule is essential for actin nucleating activity and is preceded by additional host modifications
AUTHOR(S): Kenny, Brendan
CORPORATE SOURCE: Department of Pathology and Microbiology, School of Medical Sciences, University Walk, Bristol, BS8 1TD, UK
SOURCE: Mol. Microbiol. (1999), 31(4), 1229-1241
CODEN: MOMIEE; ISSN: 0950-382X
PUBLISHER: Blackwell Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The enteropathogenic Escherichia coli (EPEC) **Tir** protein becomes tyrosine phosphorylated in host cells and displays an increase in apparent mol. mass. The interaction of **Tir** with the EPEC outer membrane protein, **intimin**, triggers actin nucleation beneath the adherent bacteria. The **enterohaemorrhagic** E. **coli** 0157:H7 (EHEC) **Tir** mol. is not tyrosine phosphorylated. In this paper, **Tir** tyrosine phosphorylation is shown to be essential for actin nucleation activity, but not for the increase in apparent mol. mass obsd. in target cells. Tyrosine phosphorylation had no role in **Tir** mol. mass shift, indicating addnl. host modifications. Anal. of **Tir** intermediates indicates that tyrosine-independent modification functions to direct **Tir** 's correct insertion from the cytoplasm into the host membrane. Deletion anal. identified **Tir** domains participating in translocation, assocn. with the host membrane, modification and antibody recognition. **Intimin** was found to bind a 55-amino-acid region (TIBA) within **Tir** that topol. and sequence anal. suggests is located in an extracellular loop. Homologous TIBA sequences exist in integrins, which also bind **intimin**. Collectively, this study provides definitive evidence for the importance of tyrosine phosphorylation for EPEC **Tir** function and reveals differences in the pathogenicity of EPEC and EHEC. The data also suggest a mechanism for **Tir** insertion into the host membrane, as well as providing clues to the mode of **intimin**-integrin interaction.

REFERENCE COUNT: 33
REFERENCE(S):
(1) Abe, A; Infect Immun 1997, V65, P3547 CAPLUS
(2) Adu-Bobie, J; J Clin Microbiol 1998, V36, P662 CAPLUS
(3) Anderson, D; Science 1997, V278, P1140 CAPLUS

Searcher : Shears 308-4994

(4) Beaulieu, J; J Cell Sci 1992, V102, P427 CAPLUS
(5) Clark, M; Infect Immun 1998, V66, P1237 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 10 OF 19 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1999:99523 CAPLUS
DOCUMENT NUMBER: 130:292160
TITLE: Detection of Shiga-like toxin (stx1 and stx2), **intimin** (**eaeA**), and **enterohemorrhagic** Escherichia **coli** (EHEC) hemolysin (EHEC hlya) genes in animal feces by multiplex PCR
AUTHOR(S): Fagan, Peter K.; Hornitzky, Michael A.; Bettelheim, Karl A.; Djordjevic, Steven P.
CORPORATE SOURCE: Elizabeth Macarthur Agricultural Institute, New South Wales Agriculture, Camden, 2570, Australia
SOURCE: Appl. Environ. Microbiol. (1999), 65(2), 868-872
CODEN: AEMIDF; ISSN: 0099-2240
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A multiplex PCR was developed for the rapid detection of genes encoding Shiga toxins 1 and 2 (stx1 and stx2), **intimin** (**eaeA**), and enterohemolysin A (hlyA) in 444 fecal samples derived from healthy and clin. affected cattle, sheep, pigs, and goats. The method involved non-solvent-based extn. of nucleic acid from an aliquot of an overnight culture of feces in EC (modified) broth. The detection limit of the assay for both fecal samples and pure cultures was between 18 and 37 genome equiv. Stx1 and hlyA were the most commonly encountered virulence factors.
REFERENCE COUNT: 32
REFERENCE(S): (2) Agin, T; FEMS Microbiol Lett 1996, V144, P249 CAPLUS
(5) Beutin, L; Appl Environ Microbiol 1997, V63, P2175 CAPLUS
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(13) Gannon, V; Appl Environ Microbiol 1992, V58, P3809 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 11 OF 19 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1997:474667 CAPLUS
DOCUMENT NUMBER: 127:173509
TITLE: Hemolysin phenotypes and genotypes of

**EAEA**-positive and **EAEA**
-negative bovine verotoxigenic Escherichia coli

AUTHOR(S): Sandhu, K. S.; Clarke, R. C.; Gyles, C. L.
CORPORATE SOURCE: Veterinary Microbiology and Immunology, University of Guelph, Guelph, ON, Can.

SOURCE: Adv. Exp. Med. Biol. (1997), 412(Mechanisms in the Pathogenesis of Enteric Diseases), 295-302
CODEN: AEMBAP; ISSN: 0065-2598
PUBLISHER: Plenum
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

AB A review and discussion with 19 refs. on the assocn. of the virulence marker, **eaeA** and several types of hemolysin (hly), with serotypes of verotoxigenic Escherichia coli isolated from cattle and implicated in human disease. **Intimin** or **EaeA** protein has been implicated in the attaching/effacing lesion caused by **entero-hemorrhagic** Escherichia **coli** (EHEC) in the intestine but it is not produced by all EHEC and is therefore not adequate as a marker for EHEC. Hemolysins are produced by a high percentage of verotoxigenic E. coli (VTEC) and could be a marker for EHEC, but their distribution and relation to virulence are not known. We used PCR amplification to det. the presence or absence of **eaeA** sequences in 281 VTEC isolates from the feces of healthy cattle. There were 101 **eaeA** -pos. isolates, which belonged to O groups 5, 26, 69, 80, 84, 98, 103, 111, 119, 145, 157 and 108 **eaeA**-neg. isolates, which belonged to O groups 8, 22, 38, 113, 119, 116, 132, 153, 156 or untypable. All isolates were tested for hemolysis on horse blood agar and on washed sheep blood agar. PCR amplification was used to test for EHEC hemolysin, Ehly1, Ehly2 and alpha-hemolysin D sequences. Among **eaeA**-pos. isolates 98% were pos. for EHEC hemolysin sequences and were hemolytic on washed sheep red blood cell agar; the corresponding percentage for **eaeA** -neg. isolates was 36%. Ehly1 and Ehly2 sequences were present in only 11 isolates (O groups 26, 84, 119 and 132). None of the **eaeA**-pos. and 13 of the **eaeA**-neg. isolates (including all 11 isolates of O group 132) were pos. for alpha-hemolysin D gene sequences by PCR and alpha-hemolysin prodn. on horse blood agar. We conclude that since Ehly1, Ehly2 and alpha-hemolysin occur at low frequency among bovine VTEC and serotypes implicated in human disease they are unlikely to be significant virulence factors. In contrast, EHEC hemolysin was present in almost all **eaeA**-pos. VTEC isolates and in approx. one-third the **eaeA**-neg. ones; it may be both a virulence marker and virulence factor but further testing is required. The study identified isolates with all combinations of **eaeA** and EHEC hly, which may be useful for further testing.

L7 ANSWER 12 OF 19 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1997:19116 CAPLUS
DOCUMENT NUMBER: 126:57335
TITLE: Identification of a family of **intimins** common to Escherichia coli causing attaching-effacing lesions in rabbits, humans, and swine
AUTHOR(S): Agin, Tonia S.; Wolf, Marcia K.
CORPORATE SOURCE: Dep. Gastroenterology, Walter Reed Army Inst. Res., Washington, DC, 20307-5100, USA
SOURCE: Infect. Immun. (1997), 65(1), 320-326
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Intimin**, an outer membrane protein encoded by **eaeA** that mediates close attachment of enteropathogenic bacteria to apical surfaces of epithelial cells, is required for formation of the attaching-effacing lesions and for full pathogenesis of the bacteria. Anal. of the **eaeA** sequence indicates that there is a high degree of homol. at the N termini but less at the C termini of **intimins**. Antisera specific for the C-terminal third of RDEC-1 **intimin**, used to screen outer membrane proteins from 50 rabbit enteropathogenic Escherichia **coli** (EPEC), human EPEC, and human **enterohemorrhagic** E. **coli** (EHEC) strains, identified cross-reactive **intimins** from 24 isolates. Sequence anal. of the **eaeA** genes from human EPEC O111 and EHEC O26 isolates indicates that their **intimins** have C termini nearly identical to that of RDEC-1 **intimin**. Our results suggest that there are at least three families of related **intimins** and that the presence of **intimin** similar to that of RDEC-1 is not restricted by serogroup or host specificity.

L7 ANSWER 13 OF 19 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1996:718948 CAPLUS
DOCUMENT NUMBER: 126:16557
TITLE: **Intimin** from enteropathogenic Escherichia coli restores murine virulence to a **Citrobacter rodentium** **eaeA** mutant: induction of an immunoglobulin A response to **intimin** and EspB
AUTHOR(S): Frankel, Gad; Phillips, Alan D.; Novakova, Michaela; Field, Helen; Candy, David C. A.; Schauer, David B.; Douce, Gill; Dougan, Gordon
CORPORATE SOURCE: Dep. Biochem., Imperial Coll. Sci. Technol. Med., London, SW7 2AZ, UK
SOURCE: Infect. Immun. (1996), 64(12), 5315-5325
CODEN: INFIBR; ISSN: 0019-9567

PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The formation of attaching and effacing (A/E) lesions is central to the pathogenesis of enteropathogenic E. coli (EPEC)-mediated disease in humans and C. rodentium (formerly C. freundii biotype 4280)-mediated transmissible colonic hyperplasia in mice. Closely related outer membrane proteins, known as **intimins**, are required for formation of the A/E lesion by both EPEC (IntEPEC) and C. rodentium (IntCR). A secreted protein, EspB (formally EaeB), is also necessary for A/E-lesion formation. Here it is reported that expression of a cloned IntEPEC, encoded by plasmid pCVD438, restores murine virulence to an **intimin**-deficient mutant of C. rodentium DBS255. Replacement of Cys937 with Ala abolished the ability of the cloned EPEC **intimin** to complement the deletion mutation in DBS255. Ultrastructural examn. of tissues from wild-type C. rodentium and DBS255 (pCVD438)-infected mice revealed multiple A/E lesion on infected cells and loss of contact between enterocytes and basement membrane. Histol. investigation showed that although both wild-type C. rodentium and DBS255 (pCVD438) colonized the descending colon and induced colonic hyperplasia in orally infected 21-day-old mice, the latter strain adhered to epithelial cells located deeper within crypts. Nonetheless, infection with the wild-type strain was consistently more virulent, as indicated by a higher mortality rate. All the surviving mice, challenged with either wild-type C. rodentium or DBS255 (pCVD438), developed a mucosal IgA response to **intimin** and EspB. These results show that C. rodentium infection provides a relevant, simple, and economic model to investigate the role of EPEC proteins in the formation of A/E lesions in vivo and in intestinal disease.

L7 ANSWER 14 OF 19 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1996:684497 CAPLUS
DOCUMENT NUMBER: 126:2069
TITLE: Characterization of the **eaeA** gene from rabbit enteropathogenic Escherichia coli strain RDEC-1 and comparison to other **eaeA** genes from bacteria that cause attaching-effacing lesions
AUTHOR(S): Agin, Tonia S.; Cantley, J. Robert; Boedeker, Edgar C.; Wolf, Marcia K.
CORPORATE SOURCE: Walter Reed Army Institute of Research, Washington, DC, 20307-5100, USA
SOURCE: FEMS Microbiol. Lett. (1996), 144(2-3), 249-258
CODEN: FMLED7; ISSN: 0378-1097
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A no. of enteric pathogens, including enteropathogenic (EPEC) and

**enterohemorrhagic** (EHEC) Escherichi **coli**, Hafnia alvie, a strain of Citrobacter freundii, and rabbit EPEC strain RDEC-1 cause attaching-effacing (AE) lesions in the gut mucosa. These bacteria have a pathogenicity cassette (locus of enterocyte effacement or LEE) contg. the **eaeA** gene. This gene encodes **intimin**, an outer membrane protein for prodn. of AE lesions. RDEC-1, a non-invasive enteropathogen in young rabbits, produces AE lesions morphol. indistinguishable from lesions caused by human AE bacterial strains. The RDEC-1 example of E. coli diarrhea in rabbits is an important model for studying the pathogenesis of AE bacteria in a natural infection and for analyzing specific roles of the components of LEE. In order to better understand the role of **intimin** in the development of AE lesions, a portion of DNA within RDEC-1 LEE, contg. the **eaeA** gene and an upstream open reading frame (ORF), was sequenced. The RDEC-1 **eaeA** gene shared 87%, 92%, and 93% DNA sequence identity and >80% amino acid sequence identity with the **eaeA** genes of C. freundii biotype 4280, EHEC O157:H7, and EPEC O126:H6, resp. The carboxy-terminal 280 amino acid residues of **intimin** 80%, 56%, and 54% identity with C. freundii, EHEC O157:H7, and EPEC O127:H6 **intimins**, resp. The predicted protein encoded by the upstream ORF (156 amino acids) shares 95%, 97%, and 99% amino acid identity with predicted proteins from C. freundii EHEC O157:H7, and EPEC O127:H6, resp. The high degree of sequence homol. of the ORF and the **eaeA** gene of RDEC-1 with those of other AE bacteria suggests an evolutionary relationship of LEE and supports and facilitates the use of the RDEC-1 model for studying the role of LEE in pathogenesis.

IT **183450-70-6**
RL: ADV (Adverse effect, including toxicity); PRP (Properties); BIOL (Biological study)
(amino acid sequence; characterization of the **eaeA** gene from rabbit enteropathogenic Escherichia coli strain RDEC-1 and comparison to other **eaeA** genes from bacteria that cause attaching-effacing lesions)

L7 ANSWER 15 OF 19 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1996:327998 CAPLUS
DOCUMENT NUMBER: 125:7144
TITLE: Truncated **enterohemorrhagic** Escherichia **coli** (EHEC) O157:H7 **intimin** (**EaeA**) fusion proteins promote adherence of EHEC strains to HEp-2 cells
AUTHOR(S): McKee, Marian L.; O'Brien, Alison D.
CORPORATE SOURCE: Department Microbiology and Immunology, F. Edward Hebert School Medicine, Bethesda, MD, 20814-4799, USA
SOURCE: Infect. Immun. (1996), 64(6), 2225-2233
CODEN: INFIBR; ISSN: 0019-9567
Searcher : Shears 308-4994

DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Intimin**, the product of the **eaeA** gene in **enterohemorrhagic** Escherichia **coli** O157:H7 (EHEC), is required for intimate adherence of these organisms to tissue culture cells and formation of the attaching and effacing lesion in the gnotiobiotic pig. Because of the importance of **intimin** in the pathogenesis of EHEC O157:H7 infection in this animal model, we began a structure-function anal. of **EaeA**. For this purpose, we constructed amino-terminal fusions of the **intimin** protein with 6 histidine residues to form two independent fusions. The longer fusion, RIHisEae, contained 900 of the 935 predicted amino acids and included all but the extreme amino terminus. The second fusion, RVHdHisEae, consisted of the carboxyl 2/3 of the protein. Purified exts. of either construct enhanced binding of wild-type 86-24 to HEp-2 cells and conferred HEp-2 cell adherence on 86-24eae.DELTA.10, an **eaeA** deletion mutant, and B2F1, an EHEC O91:H21 **eaeA** mutant strain. When 86-24eae.DELTA.10 was transformed with either of the plasmids encoding the **intimin** fusion proteins, the transformant behaved like the wild-type parent strain and displayed localized adherence to HEp-2 cells, with pos. fluorescent-actin staining. In addn., polyclonal antisera raised against RIHisEae reacted with both fusion constructs and recognized an outer membrane protein of the same mass as **intimin** (97 kDa) in EHEC and enteropathogenic E. coli but not E. coli K-12. The **intimin**-specific antisera also blocked adherence to EHEC to HEp-2 cells. Thus, **intimin** (i) is a 97-kDa outer membrane protein in EHEC that serves as a requisite adhesin for attachment of the bacteria to epithelial cells, even when the protein is truncated by 1/3 at its amino terminus and (ii) can be added exogenously to specifically facilitate HEp-2 cell adherence of EHEC but not E. coli K-12.

L7 ANSWER 16 OF 19 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1995:781469 CAPLUS
DOCUMENT NUMBER: 123:195488
TITLE: **Enterohemorrhagic** Escherichia **coli** O157:H7 requires **intimin** to colonize the gnotobiotic pig intestine and to adhere to HEp-2 cells
AUTHOR(S): McKee, Marian L.; Melton-Celsa, Angela R.; Moxley, Rodney A.; Francis, David H.; O'Brien, Alison D.
CORPORATE SOURCE: Dep. Microbiol. Immunol., F. Edward Hebert Sch. Med., Bethesda, MD, 20814-4799, USA
SOURCE: Infect. Immun. (1995), 63(9), 3739-44
CODEN: INFIBR; ISSN: 0019-9567
DOCUMENT TYPE: Journal
LANGUAGE: English

AB In a previous study, **enterohemorrhagic** Escherichia **coli** (EHEC) O157:H7 with a deletion and insertion in the **eaeA** gene encoding **intimin** was used to establish that **intimin** is required for the organism to attach to and efface microvilli in the piglet intestine. However, in the same investigation, a role for **intimin** in EHEC adherence to HEp-2 cells could not be definitively demonstrated. To analyze the basis for this discrepancy, we constructed an in-frame deletion of **eaeA** and compared the adherence capacity of this mutant with that of the wild-type strain in vitro and in vivo. We obsd. a direct correlation between the requisite for **intimin** in EHEC O157:H7 colonization of the gnotobiotic piglet intestine and adherence of the bacterium to HEp-2 cells. The in vitro-in vivo correlation lends credence to the use of the HEp-2 cell adherence model for further study of the **intimin** protein.

L7 ANSWER 17 OF 19 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1995:781448 CAPLUS
DOCUMENT NUMBER: 123:195484
TITLE: The role of the **eaeA** gene in diarrhea and neurological complications in a gnotobiotic piglet model of **enterohemorrhagic** Escherichia **coli** infection
AUTHOR(S): Tzipori, Saul; Gunzer, Florian; Donnenberg, Michael S.; de Montigny, Lina; Kaper, James B.; Donohue-Rolfe, Arthur
CORPORATE SOURCE: Div. Infectious Diseases, Tufts Univ. Sch. Veterinary Med., North Grafton, MA, USA
SOURCE: Infect. Immun. (1995), 63(9), 3621-7
CODEN: INFIBR; ISSN: 0019-9567
DOCUMENT TYPE: Journal
LANGUAGE: English
AB We reported previously that mutation of the chromosomal gene **eaeA** from **enterohemorrhagic** Escherichia **coli** (EHEC) serotype )157:H7 prevented bacterial attachment in vivo. Attachment was restored when the EHEC or enteropathogenic E. coli (EPEC) **eaeA** gene was introduced into the mutant on a plasmid. In this communication we have compared in gnotobiotic piglets the pathogenicities of wild-type O157:H7 strain 86-24 and its **eaeA** mutant UMD619 with those of the two plasmid-complemented strains expressing IntiminO157 (EHEC) and IntiminO127 (EPEC). 86-24 Colonized the surface and glandular epithelium of the large intestine and induced diarrhea, while UMD619 did not colonize any intestinal site and induced little or no diarrhea. Surprisingly, strain UMD619 expressing IntiminO127 behaved in pigs more like EPEC than EHEC strains; it colonized the distal half of the small intestine and the surface of the large intestine, inducing serious diarrhea. In contrast, strain UMD619 expressing IntiminO157 colonized the colon extremely poorly,

inducing little or no diarrhea. While only the two strains causing extensive attachment-86-24 and UMD619 expressing IntiminO127-induced diarrhea, neurol. symptoms attributed to Shiga-like toxin II occurred equally in all four groups of animals. The intimate bacterial attachment and mucosal damage were not a prerequisite for Shiga-like toxin II translocation from the gut lumen into the circulation. IntiminO127 appears not only to facilitate intimate attachment to cells but also to influence the site of intestinal colonization and other characteristics of EPEC infection.

L7 ANSWER 18 OF 19 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1995:641439 CAPLUS
DOCUMENT NUMBER: 123:51827
TITLE: Identification of **EaeA** protein in the outer membrane of attaching and effacing Escherichia coli O45 from pigs
AUTHOR(S): Zhu, Chengru; Harel, Josee; Dumas, France; Fairbrother, John M.
CORPORATE SOURCE: Groupe de Recherche sur les Maladies Infectieuses du Porc, Universite de Montreal, Faculte de Medecine Veterinaire, C.P. 5000, Saint-Hyacinthe, Can.
SOURCE: FEMS Microbiol. Lett. (1995), 129(2-3), 237-42
CODEN: FMLED7; ISSN: 0378-1097
DOCUMENT TYPE: Journal
LANGUAGE: English
AB We have previously reported that the prodn. of attaching and effacing lesions by Escherichia coli O45 isolates from pigs is assocd. with the **eaeA** (E. coli attaching and effacing) gene. In the present study, expression of the **EaeA** protein, the **eaeA** gene product, among swine O45 E. coli isolates was examd. The majority (20/22) of attaching and effacing pos., **eaeA**+ E. coli O45 isolates, but none of ten attaching and effacing neg., **eaeA**- or **eaeA**+ isolates, expressed a 97-kDa outer membrane protein as revealed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis ( **SDS-PAGE**) and Western blot anal. Amino-terminal amino acid sequencing demonstrated a high homol. between this 97-kDa protein of swine E. **coli** O45 and the **EaeA** protein (**intimin**) of human enteropathogenic E. **coli** and **enterohemorrhagic** E. **coli**. In addn., a serol. relationship between the **EaeA** proteins of swine O45, rabbit (RDEC-1) and human (E2348/69) attaching and effacing E. coli strains was obsd. Our results indicate an assocn. between expression of the **EaeA** protein and attaching and effacing activity among O45 E. coli isolates. The data also suggest an antigenic relatedness of the **EaeA** proteins of swine, rabbit, and human attaching and effacing E. coli.

L7 ANSWER 19 OF 19 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1994:72996 CAPLUS
DOCUMENT NUMBER: 120:72996
TITLE: Expression and characterization of the **eaeA** gene product of Escherichia coli serotype O157:H7
AUTHOR(S): Louie, M.; de Azavedo, J. C. S.; Handelsman, M. Y. C.; Clark, C. G.; Ally, B.; Dytoc, M.; Sherman, P.; Brunton, J.
CORPORATE SOURCE: Samuel Lunenfeld Res. Inst., Mount Sinai Hosp., Toronto, ON, M5G 1X5, Can.
SOURCE: Infect. Immun. (1993), 61(10), 4085-92
CODEN: INFIBR; ISSN: 0019-9567
DOCUMENT TYPE: Journal
LANGUAGE: English

AB In enteropathogenic Escherichia coli, the **eaeA** gene produces a 94-kDa outer membrane protein called **intimin** which has been shown to be necessary but not sufficient to produce the attaching-and-effacing lesion. This study characterizes the **intimin** specified by the **eaeA** allele of the **enterohemorrhagic** E. **coli** (EHEC) serotype O157:H7 strain CL8 and dets. its role in adherence. The C-terminal 266 amino acids of the CL8 **intimin** were expressed as a protein fusion with glutathione S-transferase, which was used to raise antiserum in rabbits. The antiserum reacted in Western immunoblots with a 97-kDa outer membrane protein of EHEC strains of serogroups O5, O26, O111, and O157 and enteropathogenic E. coli strains of serogroups O55 and O127. Surface labeling of CL8 with 125I showed that **intimin** was surface exposed. An **eaeA** insertional inactivation mutant of CL8 was produced and was designated CL8-KO1. Total adherence of CL8-KO1 to HEp-2 cells was not significantly different from that of CL8, but CL8-KO1 gave a neg. result in the fluorescent actin staining test. The **eaeA** gene expressed alone in E. coli HB101 also gave a neg. fluorescent actin staining test result. The **eaeA** gene of CL8 complemented the **eaeA** deletion mutation in CVD206. Thus, the product of the EHEC **eaeA** gene is a 97-kDa surface-exposed protein designated intiminO157. P. Sherman et al. (1991) described a 94-kDa outer membrane protein which played an important role in adherence of E. coli. Western immunoblotting and indirect fluorescent antibody studies showed that the protein described by Sherman et al. is not **intimin**.

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, JAPIO, JICST-EPLUS, TOXLIT, TOXLINE, PHIC, PHIN' ENTERED AT 15:26:09 ON 26 JUL 2000)
L8 58 S L7
L9 24 DUP REM L8 (34 DUPLICATES REMOVED)

L9 ANSWER 1 OF 24 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 2000186699 EMBASE
TITLE: Structural basis for recognition of the **translocated intimin** receptor (**Tir**) by **intimin** from enteropathogenic Escherichia coli.
AUTHOR: Batchelor M.; Prasannan S.; Daniell S.; Reece S.; Connerton I.; Bloomberg G.; Dougan G.; Frankel G.; Matthews S.
CORPORATE SOURCE: S. Matthews, Department of Biochemistry, Imp. Coll. Sci., Technol. Medicine, London SW7 2AZ, United Kingdom. s.j.matthews@ic.ac.uk
SOURCE: EMBO Journal, (1 Jun 2000) 19/11 (2452-2464). Refs: 59
ISSN: 0261-4189 CODEN: EMJODG
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB **Intimin** is a bacterial adhesion molecule involved in intimate attachment of enteropathogenic and **enterohaemorrhagic** Escherichia **coli** to mammalian host cells. **Intimin** targets the **translocated intimin** receptor (**Tir**), which is exported by the bacteria and integrated into the host cell plasma membrane. In this study we localized the **Tir**-binding region of **intimin** to the C-terminal 190 amino acids (Int190). We have also determined the region's high-resolution solution structure, which comprises an immunoglobulin domain that is intimately coupled to a novel C-type lectin domain. This fragment, which is necessary and sufficient for **Tir** interaction, defines a new super domain in **intimin** that exhibits striking structural similarity to the integrin-binding domain of the Yersinia invasin and C-type lectin families. The extracellular portion of **intimin** comprises an articulated rod of immunoglobulin domains extending from the bacterium surface, conveying a highly accessible 'adhesive tip' to the target cell. The interpretation of NMR-titration and mutagenesis data has enabled us to identify, for the first time, the binding site for **Tir**, which is located at the extremity of the Int190 moiety.

L9 ANSWER 2 OF 24 WPIDS COPYRIGHT 2000 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1999-540860 [45] WPIDS
DOC. NO. NON-CPI: N1999-400815
DOC. NO. CPI: C1999-158073
TITLE: Identifying antibacterial agents that inhibit the Gram-negative type III secretion system, for treating infections - by screening for inhibition of virulence factors secreted by this system.
Searcher : Shears 308-4994

DERWENT CLASS: B04 D16 S03
INVENTOR(S): FINLAY, B B; KENNY, B; STEIN, M
PATENT ASSIGNEE(S): (UYBR-N) UNIV BRITISH COLUMBIA
COUNTRY COUNT: 83
PATENT INFORMATION:

| PATENT NO | KIND | DATE | WEEK | LA | PG |
|---|---|---|---|---|---|
| WO 9945136 | A1 | 19990910 | (199945)* | EN | 51 |
| RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ UG ZW | | | | | |
| W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG UZ VN YU ZW | | | | | |
| AU 9932431 | A | 19990920 | (200007) | | |

APPLICATION DETAILS:

| PATENT NO | KIND | APPLICATION | DATE |
|---|---|---|---|
| WO 9945136 | A1 | WO 1999-CA183 | 19990305 |
| AU 9932431 | A | AU 1999-32431 | 19990305 |

FILING DETAILS:

| PATENT NO | KIND | | PATENT NO |
|---|---|---|---|
| AU 9932431 | A | Based on | WO 9945136 |

PRIORITY APPLN. INFO: US 1998-76980 19980305
AN 1999-540860 [45] WPIDS
AB WO 9945136 A UPAB: 19991103
NOVELTY - Identification of antibacterial agents (I) comprises:
(i) treating bacteria that contain a polynucleotide (II) which encodes a polypeptide (III) secreted by the type III secretion system (3SS) with a test compound and
(ii) detecting secretion of (III).
A reduction of secretion, relative to that in bacteria not treated with the test compound, indicates an inhibitor of 3SS.
DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a kit containing, in separate containers, the bacteria and a system for detecting secretion of (III).
ACTIVITY - Antibacterial.
MECHANISM OF ACTION - (I) blocks the 3SS which is used to secrete virulence factors essential for pathogenicity.
USE - (I) are used:
(i) to treat bacterial infections in humans, other animals and plants, e.g. where caused by enteropathogenic or

**enterohemorrhagic** Escherichia **coli**, Yersinia species, Shigella species, Pseudomonas aeruginosa, P. syringae, Xanthomonas campestris, or many others listed;

(ii) for analyzing the functional mechanisms of 3SS (which involves about 20 gene products), and

(iii) for development of more powerful or specific inhibitors.

ADVANTAGE - 3SS is conserved in many Gram-negative bacteria, so (I) should have a broad spectrum of activity, and is used exclusively to secrete virulence factors. It is absent from non-pathogenic bacteria. The method can identify inhibitors specific for 3SS.

Dwg.0/2

L9 ANSWER 3 OF 24 WPIDS COPYRIGHT 2000 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1999-540157 [45] WPIDS
DOC. NO. NON-CPI: N1999-400313
DOC. NO. CPI: C1999-157755
TITLE: Detecting microorganisms that express **intimin**, for diagnosis of infection, e.g. by enteropathogenic Escherichia coli.
DERWENT CLASS: B04 D13 D16 J04 S03
INVENTOR(S): BATCHELOR, M; DOUGAN, G; FRANKEL, G
PATENT ASSIGNEE(S): (UNLO) IMPERIAL COLLEGE SCI TECHNOLOGY & MED
COUNTRY COUNT: 83
PATENT INFORMATION:

| PATENT NO | KIND | DATE | WEEK | LA | PG |
|---|---|---|---|---|---|
| WO 9941614 | A2 | 19990819 | (199945)* | EN | 56 |
| RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW | | | | | |
| W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW | | | | | |
| AU 9925356 | A | 19990830 | (200003) | | |

APPLICATION DETAILS:

| PATENT NO | KIND | APPLICATION | DATE |
|---|---|---|---|
| WO 9941614 | A2 | WO 1999-GB467 | 19990216 |
| AU 9925356 | A | AU 1999-25356 | 19990216 |

FILING DETAILS:

| PATENT NO | KIND | | PATENT NO |
|---|---|---|---|
| AU 9925356 | A | Based on | WO 9941614 |

Searcher : Shears 308-4994

PRIORITY APPLN. INFO: GB 1998-3322 19980216
AN 1999-540157 [45] WPIDS
AB WO 9941614 A UPAB: 19991103
NOVELTY - Microorganisms (A) that express **intimin** (I) are detected either from their reaction with antisera raised against (I) or by PCR amplification of DNA with (I)-specific primers.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:
(1) isolated and recombinant polypeptide (II) comprising the Gly387 to Lys666 region of the **eae** protein from enteropathogenic or enterohemorrhagic microorganisms, or fragments of (II);
(2) nucleic acd (III) encoding (II);
(3) vector containing (III);
(4) host cell transformed with (III) or the vector;
(5) primers that hybridize to **intimin** alpha , beta , gamma or delta nucleic acid;
(6) antisera raised against the EPEC (enteropathogenic Escherichia coli) serotypes O127:H6 or O114:H2, or against (II);
(7) kits for detecting (A);
(8) agent for isolating (A) comprising antibodies (Ab) raised against (I); and
(9) vaccine containing (II) or (III).
ACTIVITY - Antibacterial; antidiarrhoea.
MECHANISM OF ACTION - Induction of a specific immune response.
USE - The methods are used to detect (A) in foods; to classify or type (A) and to diagnose infections caused by (A), specifically enteropathogenic (EPEC) or **enterohemorrhagic** (EHEC) strains of Escherichia **coli**; **Citrobacter rodentium** and/or rabbit diarrheogenic E. **coli**, which are causative agents of diarrhea, hemolytic uremic syndrome and colonic hyperplasia. Antibodies (Ab) raised against (I) are also used to isolate (A) (by immunoaffinity) and fragmens of (II), or nucleic acid encoding them, are used in vaccines to prevent and/or treat infections by (A).
Dwg.0/3

L9 ANSWER 4 OF 24 WPIDS COPYRIGHT 2000 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1999-337712 [28] WPIDS
DOC. NO. NON-CPI: N1999-253081
DOC. NO. CPI: C1999-099316
TITLE: New **translocated intimin** receptor useful for treating infection by enteropathogenic or **enterohemorrhagic** Escherichia **coli**.
DERWENT CLASS: B04 D16 S03
INVENTOR(S): DEVINNEY, R; FINLAY, B B; KENNY, B; STEIN, M
PATENT ASSIGNEE(S): (UYBR-N) UNIV BRITISH COLUMBIA
Searcher : Shears 308-4994

COUNTRY COUNT: 81
PATENT INFORMATION:

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| AU 9911373 | A | 19990531 | (199941) | | |

APPLICATION DETAILS:

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| WO 9924576 | A1 | WO 1998-CA1042 | 19981110 |
| AU 9911373 | A | AU 1999-11373 | 19981110 |

FILING DETAILS:

| PATENT NO | KIND | | PATENT NO |
|---|---|---|---|
| AU 9911373 | A | Based on | WO 9924576 |

PRIORITY APPLN. INFO: US 1997-65130 19971112
AN 1999-337712 [28] WPIDS
AB WO 9924576 A UPAB: 19990719
NOVELTY - A **translocated intimin** receptor ( **Tir**) polypeptide that binds **intimin**, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:
(1) an isolated polynucleotide (I) encoding **Tir**;
(2) a polynucleotide selected from:
(a) the 1920 bp sequence (Ia) given in the specification;
(b) (Ia) where T is U;
(c) nucleic acid sequences complementary to (a) or (b);
(d) fragments of (a), (b) or (c) that are at least 15 nucleotides long and that hybridize to DNA which encode the 549 amino acid polypeptide defined in the specification;
(3) a polynucleotide selected from:
(a) the 1723 bp sequence (Ib) given in the specification;
(b) (Ib) where T is U;
(c) nucleic acid sequences complementary to (a) or (b);
(d) fragments of (a), (b) or (c) that are at least 15 nucleotides long and that hybridize to DNA which encode the 559 amino acid polypeptide defined in the specification;

(4) a vector containing (I);
(5) a host cell containing the vector of (4);
(6) an anti-**Tir** antibody;
(7) detecting **Tir** or its polynucleotides in a sample comprising:
(a) contacting the sample with an anti-**Tir** antibody or a nucleic acid probe that hybridizes to the **Tir** polynucleotide;
(b) detecting binding of the antibody to **Tir** polypeptide, where binding is indicative of the presence of the **Tir** polypeptide in the sample; or hybridization of the probe with the **Tir** polynucleotide which is indicative of **Tir** polynucleotide in the sample;
(8) a recombinant method for the production of **Tir** polynucleotides and polypeptides;
(9) a polynucleotide produced by (8);
(10) a host cell containing the polynucleotide of (9);
(11) production of a **Tir** fusion protein;
(12) identifying a compound that interferes with binding of **Tir** to **intimin** comprises comparing the binding of the **Tir** polypeptide to **intimin** in the presence and absence of the compound ;
(13) a method for differentiating among attaching and effacing pathogens by contacting them with an anti-**Tir** antibody and an anti-phosphotyrosine antibody;
(14) delivering a compound of interest to a **Tir** -containing cell by administering to the cell an **intimin** -containing cell delivery vehicle that contains a compound of interest;
(15) kits for detection of **Tir** polypeptides or polynucleotides; and
(16) a method for inducing a cell-mediated immune response to a polypeptide of interest, by contacting a subject with an attenuated bacteria, where the bacteria lacks an EspA or EspB protein and where the bacteria contains a polynucleotide encoding a **Tir** fusion protein.

ACTIVITY - Antibacterial.
MECHANISM OF ACTION - Vaccine.
USE - Tir antibodies can be used to detect Tir in tissue or biological fluids, where presence of Tir is indicative of infection by enteropathogenic or enterohemorrhagic Escherichia coli (designated EPEC and EHEC, respectively). The antibody is able to differentiate among attaching and effacing pathogens, when used in conjunction with an anti-phosphotyrosine antibody. Tir can be used to induce an immune response in humans or cows against EPEC or EHEC to ameliorate diseases caused by the Tir-producing EPEC or EHEC. Tir polynucleotides can be used as probes to detect the presence of Tir polynucleotides in a sample. Tir can also be used to detect a cell cytoskeleton. Additionally, Tir can be used to identify compounds

that interfere with Tir binding to intimin. The Tir fusion proteins can be used in attenuated Escherichia coli to induce a cell-mediated immune response to polypeptides of interest, e.g. antigens (all Claimed).
Dwg.0/9

L9 ANSWER 5 OF 24 TOXLIT
ACCESSION NUMBER: 1999:23044 TOXLIT
DOCUMENT NUMBER: CA-130-333761K
TITLE: Pathogenic Escherichia coli **intimin** receptor **Tir** and gene **tir** and methods for detecting gene **tir** or **Tir** protein and for drug screening.
AUTHOR: Finlay BB; Kenny B; Devinney R; Stein M
SOURCE: (1999). PCT Int. Appl. PATENT NO. 9924576 05/20/1999 (University of British Columbia).
CODEN: PIXXD2.
PUB. COUNTRY: CANADA
DOCUMENT TYPE: Patent
FILE SEGMENT: CA
LANGUAGE: English
OTHER SOURCE: CA 130:333761
ENTRY MONTH: 199906
AB A polypeptide, called **Tir** (for **translocated intimin** receptor), which is secreted by attaching and effacing pathogens, such as the enteropathogenic (EPEC) and **enterohemorrhagic** (EHEC) E. **coli** is disclosed. These bacterial pathogens insert their own receptors into mammalian cell surfaces, to which the bacterial pathogen then adheres to trigger addnl. host signaling events and actin nucleation. Diagnosis of disease caused by pathogenic E. **coli** can be performed by the use of antibodies which bind to **Tir** to detect the protein or the use of nucleic acid probes for detection of nucleic acids encoding **Tir** polypeptide. Isolated nucleic acid sequences encoding **Tir** polypeptide, **Tir** peptides, a recombinant method for producing recombinant **Tir**, antibodies which bind to **Tir**, and a kit for the detection of **Tir**-producing E. **coli** are provided. A method of immunizing a host with **Tir** to induce a protective immune response to **Tir** or a second polypeptide of interest is also provided. A method for screening for compds. which interfere with the binding of bacterial pathogens to their receptors is further provided. Thus, protein Hp90, previously believed to be a host membrane protein, has been identified as an EHEC- or EPEC-secreted protein which acts as an **intimin** receptor. Proteins encoded by the espA and espB genes were necessary for delivery of **Tir** to the host membrane.

L9 ANSWER 6 OF 24 MEDLINE DUPLICATE 1
Searcher : Shears 308-4994

ACCESSION NUMBER: 2000079630 MEDLINE
DOCUMENT NUMBER: 20079630
TITLE: Quorum sensing controls expression of the type III secretion gene transcription and protein secretion in **enterohemorrhagic** and enteropathogenic Escherichia **coli**.
AUTHOR: Sperandio V; Mellies J L; Nguyen W; Shin S; Kaper J B
CORPORATE SOURCE: Center for Vaccine Development, Department of Microbiology, University of Maryland School of Medicine, 685 West Baltimore Street, Baltimore, MD 21201, USA.
CONTRACT NUMBER: AI41325 (NIAID)
AI21657 (NIAID)
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1999 Dec 21) 96 (26) 15196-201.
Journal code: PV3. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 200003
ENTRY WEEK: 20000305

AB **Enterohemorrhagic** Escherichia **coli** O157:H7 and enteropathogenic E. **coli** cause a characteristic histopathology in intestinal cells known as attaching and effacing. The attaching and effacing lesion is encoded by the Locus of Enterocyte Effacement (LEE) pathogenicity island, which encodes a type III secretion system, the **intimin** intestinal colonization factor, and the **translocated intimin** receptor protein that is translocated from the bacterium to the host epithelial cells. Using lacZ reporter gene fusions, we show that expression of the LEE operons encoding the type III secretion system, **translocated intimin** receptor, and **intimin** is regulated by quorum sensing in both **enterohemorrhagic** E. **coli** and enteropathogenic E. **coli**. The luxS gene recently shown to be responsible for production of autoinducer in the Vibrio harveyi and E. **coli** quorum-sensing systems is responsible for regulation of the LEE operons, as shown by the mutation and complementation of the luxS gene. Regulation of intestinal colonization factors by quorum sensing could play an important role in the pathogenesis of disease caused by these organisms. These results suggest that intestinal colonization by E. **coli** O157:H7, which has an unusually low infectious dose, could be induced by quorum sensing of signals produced by nonpathogenic E. **coli** of the normal intestinal flora.

L9 ANSWER 7 OF 24 MEDLINE DUPLICATE 2
Searcher : Shears 308-4994

ACCESSION NUMBER: 1999242825 MEDLINE
DOCUMENT NUMBER: 99242825
TITLE: **Enterohemorrhagic** Escherichia **coli** O157:H7 produces **Tir**, which is translocated to the host cell membrane but is not tyrosine phosphorylated.
AUTHOR: DeVinney R; Stein M; Reinscheid D; Abe A; Ruschkowski S; Finlay B B
CORPORATE SOURCE: Biotechnology Laboratory, University of British Columbia, Vancouver, British Columbia V6T 1Z3, Canada.
SOURCE: INFECTION AND IMMUNITY, (1999 May) 67 (5) 2389-98. Journal code: GO7. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199907
ENTRY WEEK: 19990704

AB Intimate attachment to the host cell leading to the formation of attaching and effacing (A/E) lesions is an essential feature of **enterohemorrhagic** Escherichia **coli** (EHEC) O157:H7 pathogenesis. In a related pathogen, enteropathogenic E. **coli** (EPEC), this activity is dependent upon translocation of the **intimin** receptor, **Tir**, which becomes tyrosine phosphorylated within the host cell membrane. In contrast, the accumulation of tyrosine-phosphorylated proteins beneath adherent EHEC bacteria does not occur, leading to questions about whether EHEC uses a **Tir**-based mechanism for adherence and A/E lesion formation. In this report, we demonstrate that EHEC produces a functional **Tir** that is inserted into host cell membranes, where it serves as an **intimin** receptor. However, unlike in EPEC, in EHEC **Tir** is not tyrosine phosphorylated yet plays a key role in both bacterial adherence to epithelial cells and pedestal formation. EHEC, but not EPEC, was unable to synthesize **Tir** in Luria-Bertani medium but was able to secrete **Tir** into M9 medium, suggesting that **Tir** synthesis and secretion may be regulated differently in these two pathogens. EHEC **Tir** and EPEC **Tir** both bind **intimin** and focus cytoskeletal rearrangements, indicating that tyrosine phosphorylation is not needed for pedestal formation. EHEC and EPEC **intimins** are functionally interchangeable, but EHEC **Tir** shows a much greater affinity for EHEC **intimin** than for EPEC **intimin**. These findings highlight some of the differences and similarities between EHEC and EPEC virulence mechanisms, which can be exploited to further define the molecular basis of pedestal formation.

L9 ANSWER 8 OF 24 MEDLINE DUPLICATE 3
Searcher : Shears 308-4994

ACCESSION NUMBER: 1999195823 MEDLINE
DOCUMENT NUMBER: 99195823
TITLE: Phosphorylation of tyrosine 474 of the enteropathogenic Escherichia coli (EPEC) **Tir** receptor molecule is essential for actin nucleating activity and is preceded by additional host modifications.
AUTHOR: Kenny B
CORPORATE SOURCE: Department of Pathology and Microbiology, School of Medical Sciences, Bristol, UK.. B.Kenny@bristol.ac.uk
SOURCE: MOLECULAR MICROBIOLOGY, (1999 Feb) 31 (4) 1229-41. Journal code: MOM. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199909
ENTRY WEEK: 19990903

AB The enteropathogenic Escherichia **coli** (EPEC) **Tir** protein becomes tyrosine phosphorylated in host cells and displays an increase in apparent molecular mass. The interaction of **Tir** with the EPEC outer membrane protein, **intimin**, triggers actin nucleation beneath the adherent bacteria. The **enterohaemorrhagic** E. **coli** 0157:H7 (EHEC) **Tir** molecule is not tyrosine phosphorylated. In this paper, **Tir** tyrosine phosphorylation is shown to be essential for actin nucleation activity, but not for the increase in apparent molecular mass observed in target cells. Tyrosine phosphorylation had no role in **Tir** molecular mass shift, indicating additional host modifications. Analysis of **Tir** intermediates indicates that tyrosine-independent modification functions to direct **Tir**'s correct insertion from the cytoplasm into the host membrane. Deletion analysis identified **Tir** domains participating in translocation, association with the host membrane, modification and antibody recognition. **Intimin** was found to bind a 55-amino-acid region (TIBA) within **Tir** that topological and sequence analysis suggests is located in an extracellular loop. Homologous TIBA sequences exist in integrins, which also bind **intimin**. Collectively, this study provides definitive evidence for the importance of tyrosine phosphorylation for EPEC **Tir** function and reveals differences in the pathogenicity of EPEC and EHEC. The data also suggest a mechanism for **Tir** insertion into the host membrane, as well as providing clues to the mode of **intimin** -integrin interaction.

L9 ANSWER 9 OF 24 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 1999124640 MEDLINE
DOCUMENT NUMBER: 99124640

TITLE: Detection of shiga-like toxin (stx1 and stx2), **intimin** (**eaeA**), and **enterohemorrhagic** Escherichia **coli** (EHEC) hemolysin (EHEC hlyA) genes in animal feces by multiplex PCR.

AUTHOR: Fagan P K; Hornitzky M A; Bettelheim K A; Djordjevic S P

CORPORATE SOURCE: Elizabeth Macarthur Agricultural Institute, New South Wales Agriculture, Camden, New South Wales 2570, Australia.

SOURCE: APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (1999 Feb) 65 (2) 868-72.
Journal code: 6K6. ISSN: 0099-2240.

PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199905

ENTRY WEEK: 19990504

AB A multiplex PCR was developed for the rapid detection of genes encoding Shiga toxins 1 and 2 (stx1 and stx2), **intimin** (**eaeA**), and enterohemolysin A (hlyA) in 444 fecal samples derived from healthy and clinically affected cattle, sheep, pigs, and goats. The method involved non-solvent-based extraction of nucleic acid from an aliquot of an overnight culture of feces in EC (modified) broth. The detection limit of the assay for both fecal samples and pure cultures was between 18 and 37 genome equivalents. stx1 and hlyA were the most commonly encountered virulence factors.

L9 ANSWER 10 OF 24 MEDLINE DUPLICATE 5

ACCESSION NUMBER: 1999348501 MEDLINE

DOCUMENT NUMBER: 99348501

TITLE: Role of bacterial **intimin** in colonic hyperplasia and inflammation.

AUTHOR: Higgins L M; Frankel G; Connerton I; Goncalves N S; Dougan G; MacDonald T T

CORPORATE SOURCE: Department of Paediatric Gastroenterology, St. Bartholomews and the Royal London School of Medicine and Dentistry, London EC1A 7BE, UK.

SOURCE: SCIENCE, (1999 Jul 23) 285 (5427) 588-91.
Journal code: UJ7. ISSN: 0036-8075.

PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals; Cancer Journals

ENTRY MONTH: 199910

ENTRY WEEK: 19991002

AB Enteropathogenic Escherichia coli (EPEC) cells adhere to gut epithelial cells through **intimin** alpha: the ligand for a

bacterially derived epithelial transmembrane protein called the **translocated intimin** receptor. **Citrobacter rodentium** colonizes the mouse colon in a similar fashion and uses a different **intimin**: **intimin** beta. **Intimin** alpha was found to costimulate submitogenic signals through the T cell receptor. Dead **intimin** beta+ C. rodentium, **intimin** alpha-transfected C. rodentium or E. coli strain K12, and EPEC induced mucosal hyperplasia identical to that caused by C. rodentium live infection, as well as a massive T helper cell-type 1 immune response in the colonic mucosa. Mutation of cysteine-937 of **intimin** to alanine reduced costimulatory activity in vitro and prevented immunopathology in vivo. The mucosal changes elicited by C. rodentium were interferon-gamma-dependent. Immunopathology induced by **intimin** enables the bacteria to promote conditions that are favorable for increased microbial colonization.

L9 ANSWER 11 OF 24 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 2000010115 MEDLINE
DOCUMENT NUMBER: 20010115
TITLE: The **Tir**-binding region of **enterohaemorrhagic** Escherichia **coli** **intimin** is sufficient to trigger actin condensation after bacterial-induced host cell signalling.
AUTHOR: Liu H; Magoun L; Luperchio S; Schauer D B; Leong J M
CORPORATE SOURCE: Department of Molecular Genetics and Microbiology, University of Massachusetts Medical Center, 55 Lake Avenue North, Worcester, MA 01655, USA.
CONTRACT NUMBER: ES07020 (NIEHS)
1S10 RR05734-01 (NCRR)
SOURCE: MOLECULAR MICROBIOLOGY, (1999 Oct) 34 (1) 67-81.
Journal code: MOM. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200002
ENTRY WEEK: 20000204
AB **Enterohaemorrhagic** Escherichia **coli** (EHEC) has emerged as an important agent of diarrhoeal disease. Attachment to host cells, an essential step during intestinal colonization by EHEC, is associated with the formation of a highly organized cytoskeletal structure containing filamentous actin, termed an attaching and effacing (A/E) lesion, directly beneath bound bacteria. The outer membrane protein **intimin** is required for the formation of this structure, as is **Tir**, a bacterial protein that is translocated into the host cell and is thought to function as a receptor for **intimin**. To

understand **intimin** function better, we fused EHEC **intimin** to a homologous protein, Yersinia pseudotuberculosis invasin, or to maltose-binding protein. The N-terminal 539 amino acids of **intimin** were sufficient to promote outer membrane localization of the C-terminus of invasin and, conversely, the N-terminal 489 amino acids of invasin were sufficient to promote the localization of the C-terminus of **intimin**. The C-terminal 181 residues of **intimin** were sufficient to bind mammalian cells that had been preinfected with an enteropathogenic E. **coli** strain that expresses **Tir** but not **intimin**. Binding of **intimin** derivatives to preinfected cells correlated with binding to recombinant **Tir** protein. Finally, the 181-residue minimal **Tir**-binding region of **intimin**, when purified and immobilized on latex beads, was sufficient to trigger A/E lesions on preinfected mammalian cells.

L9 ANSWER 12 OF 24 TOXLINE
ACCESSION NUMBER: 1999:48615 TOXLINE
DOCUMENT NUMBER: CRISP-99-AI41325-03
TITLE: NOVEL PROTEINS SECRETED BY E COLI 0157:H7.
AUTHOR: KAPER J B
CORPORATE SOURCE: UNIV OF MARYLAND SCH OF MED, 511 W LOMBARD STREET, BALTIMORE, MD 21201
U.S. DEPT. OF HEALTH AND HUMAN SERVICES; PUBLIC HEALTH SERVICE; NATIONAL INST. OF HEALTH, NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES.
CONTRACT NUMBER: 5R01AI41325-03
SOURCE: (1998). Crisp Data Base National Institutes Of Health. Award Type: G = Grant
PUB. COUNTRY: United States
DOCUMENT TYPE: (RESEARCH)
FILE SEGMENT: CRISP
LANGUAGE: English
ENTRY MONTH: 199904
AB RPROJ/CRISP DESCRIPTION The major virulence factor of **enterohemorrhagic** Escherichia **coli** 0157:H7 is a family of potent cytotoxins known as Shiga toxin, Shiga-like toxin or verocytotoxin. Data from a variety of in vitro, animal, and human studies support the concept that Shiga toxins are crucial for the development of the HUS and hemorrhagic colitis caused by this pathogen. However, there are few data concerning other potential virulence factors expressed by E. **coli** 0157:H7. Previous work in this laboratory has identified an outer membrane protein known as **intimin**, encoded by the **eaeA** gene, which is involved in intestinal colonization by this pathogen. We have recently discovered several bacterial proteins that are secreted into the culture supernatant. These proteins, which are not Shiga toxins and are not produced by non-pathogenic E.

**coli**, produce a strong serum antibody response in patients with HUS. We hypothesize that these proteins play an important role in the pathogenesis of disease due to E. **coli** 0157:H7. A multi-faceted approach is proposed to investigate these proteins. The genes encoding these proteins will be cloned and sequenced and the distribution of these genes among other Shiga toxin-producing E. **coli** will be studied. An ELISA assay will be developed for serodiagnosis and seroepidemiology studies and a large collection of well-characterized patient sera will be screened with this ELISA. Correlations of protein secretion patterns, patient immune response patterns and clinical outcome will be sought to study whether these proteins or the response to them can be used as a marker for clinical outcome. Using purified proteins and isogenic E. **coli** 0157:H7 strains specifically mutated in genes encoding these proteins, the effect of these proteins on cultured intestinal and renal cells will be studied and potential interactions with Shiga toxin will be examined in these in vitro systems. Isogenic strains mutated in these genes will also be studied in animal models to examine the contribution of these proteins to intestinal and renal manifestations of disease due to E. **coli** 0157:H7. Together, these studies should yield a more complete picture of disease due to EHEC and offer the possibility of new insights into pathogenesis, improved serodiagnostic methodologies, and identification of potential protective antigens that may be used for development of vaccines or immunotherapeutic agents.

L9 ANSWER 13 OF 24 TOXLINE
ACCESSION NUMBER: 1998:56953 TOXLINE
DOCUMENT NUMBER: CRISP-98-AI41325-02
TITLE: NOVEL PROTEINS SECRETED BY E COLI 0157:H7.
AUTHOR: KAPER J B
CORPORATE SOURCE: UNIV OF MARYLAND SCH OF MED, 511 W LOMBARD STREET, BALTIMORE, MD 21201
U.S. DEPT. OF HEALTH AND HUMAN SERVICES; PUBLIC HEALTH SERVICE; NATIONAL INST. OF HEALTH, NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES.
CONTRACT NUMBER: 5R01AI41325-02
SOURCE: (1997). Crisp Data Base National Institutes Of Health. Award Type: G = Grant
PUB. COUNTRY: United States
DOCUMENT TYPE: (RESEARCH)
FILE SEGMENT: CRISP
LANGUAGE: English
ENTRY MONTH: 199805
AB RPROJ/CRISP DESCRIPTION The major virulence factor of **enterohemorrhagic** Escherichia **coli** 0157:H7 is a family of potent cytotoxins known as Shiga toxin, Shiga-like toxin or verocytotoxin. Data from a variety of in vitro, animal, and human studies support the concept that Shiga toxins are crucial for

the development of the HUS and hemorrhagic colitis caused by this pathogen. However, there are few data concerning other potential virulence factors expressed by E. **coli** 0157:H7. Previous work in this laboratory has identified an outer membrane protein known as **intimin**, encoded by the **eaeA** gene, which is involved in intestinal colonization by this pathogen. We have recently discovered several bacterial proteins that are secreted into the culture supernatant. These proteins, which are not Shiga toxins and are not produced by non-pathogenic E. **coli**, produce a strong serum antibody response in patients with HUS. We hypothesize that these proteins play an important role in the pathogenesis of disease due to E. **coli** 0157:H7. A multi-faceted approach is proposed to investigate these proteins. The genes encoding these proteins will be cloned and sequenced and the distribution of these genes among other Shiga toxin-producing E. **coli** will be studied. An ELISA assay will be developed for serodiagnosis and seroepidemiology studies and a large collection of well-characterized patient sera will be screened with this ELISA. Correlations of protein secretion patterns, patient immune response patterns and clinical outcome will be sought to study whether these proteins or the response to them can be used as a marker for clinical outcome. Using purified proteins and isogenic E. **coli** 0157:H7 strains specifically mutated in genes encoding these proteins, the effect of these proteins on cultured intestinal and renal cells will be studied and potential interactions with Shiga toxin will be examined in these in vitro systems. Isogenic strains mutated in these genes will also be studied in animal models to examine the contribution of these proteins to intestinal and renal manifestations of disease due to E. **coli** 0157:H7. Together, these studies should yield a more complete picture of disease due to EHEC and offer the possibility of new insights into pathogenesis, improved serodiagnostic methodologies, and identification of potential protective antigens that may be used for development of vaccines or immunotherapeutic agents.

L9 ANSWER 14 OF 24 MEDLINE
ACCESSION NUMBER: 97241525 MEDLINE
DOCUMENT NUMBER: 97241525
TITLE: Genes involved in the virulence of **enterohemorrhagic** Escherichia **coli**.
AUTHOR: Izumiya H; Watanabe H
CORPORATE SOURCE: Department of Bacteriology, National Institute of Health, Japan.
SOURCE: NIPPON RINSHO. JAPANESE JOURNAL OF CLINICAL MEDICINE, (1997 Mar) 55 (3) 641-5. Ref: 16
Journal code: KIM. ISSN: 0047-1852.
PUB. COUNTRY: Japan
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
Searcher : Shears 308-4994

(REVIEW, TUTORIAL)
LANGUAGE: Japanese
ENTRY MONTH: 199708
ENTRY WEEK: 19970803
AB Some of the virulent genes of **enterohemorrhagic** E. **coli**(EHEC) are described. stx, bfpA, **eaeA** and ehxA encode an enterocytotoxin homologous to Shiga toxin, bundle-forming pilus, **intimin** and an RTX toxin, respectively. In pathogenesis, although bfp and eae cause diarrhea, and ehx and stx seem to cause hemorrhagic colitis and hemolytic uremic syndrome, many problems remain for prevention and treatment of these symptoms. In evolution, it is intriguining that the virulent genes of EHEC might be mediated by phages, plasmids and so on, because stx is derived from a bacterio-phage, bfpA is located in a plasmid in enteropathogenic E. **coli**(EPEC) though it has not yet been cloned in EHEC, **eaeA** is one of the genes in LEE(locus of enterocyte effacement), a putative genetic cassette ubiquitous for EPEC and EHEC, and ehxA is in a plasmid.

L9 ANSWER 15 OF 24 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 97130056 MEDLINE
DOCUMENT NUMBER: 97130056
TITLE: Identification of a family of **intimins** common to Escherichia coli causing attaching-effacing lesions in rabbits, humans, and swine.
AUTHOR: Agin T S; Wolf M K
CORPORATE SOURCE: Department of Gastroenterology, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100, USA.
SOURCE: INFECTION AND IMMUNITY, (1997 Jan) 65 (1) 320-6. Journal code: GO7. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
OTHER SOURCE: GENBANK-U60002; GENBANK-U59502; GENBANK-M34051; GENBANK-Z11541; GENBANK-L29509; GENBANK-L11691; GENBANK-U62655; GENBANK-U62656; GENBANK-U62657
ENTRY MONTH: 199704
ENTRY WEEK: 19970402
AB **Intimin**, an outer membrane protein encoded by **eaeA** that mediates close attachment of enteropathogenic bacteria to apical surfaces of epithelial cells, is required for formation of the attaching-effacing lesions and for full pathogenesis of the bacteria. Analysis of the **eaeA** sequence indicates that there is a high degree of homology at the N termini but less at the C termini of **intimins**. Antisera specific for the C-terminal third of RDEC-1 **intimin**, used to screen outer membrane proteins from 50 rabbit enteropathogenic Escherichia

**coli** (EPEC), human EPEC, and human **enterohemorrhagic** E. **coli** (EHEC) strains, identified cross-reactive **intimins** from 24 isolates. Sequence analysis of the **eaeA** genes from human EPEC O111 and EHEC O26 isolates indicates that their **intimins** have C termini nearly identical to that of RDEC-1 **intimin**. Our results suggest that there are at least three families of related **intimins** and that the presence of **intimin** similar to that of RDEC-1 is not restricted by serogroup or host specificity.

L9 ANSWER 16 OF 24 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 97335367 MEDLINE
DOCUMENT NUMBER: 97335367
TITLE: Hemolysin phenotypes and genotypes of **eaeA** -positive and **eaeA**-negative bovine verotoxigenic Escherichia coli.
AUTHOR: Sandhu K S; Clarke R C; Gyles C L
CORPORATE SOURCE: University of Guelph, ON, Canada.
SOURCE: ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY, (1997) 412 295-302.
Journal code: 2LU. ISSN: 0065-2598.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199710
ENTRY WEEK: 19971005
AB **Intimin** or **EaeA** protein has been implicated in the attaching/effacing lesion caused by **entero-hemorrhagic** Escherichia **coli** (EHEC) in the intestine but it is not produced by all EHEC and is therefore not adequate as a marker for EHEC. Hemolysins are produced by a high percentage of verotoxigenic E. **coli** (VTEC) and could be a marker for EHEC, but their distribution and relation to virulence are not known. We used PCR amplification to determine the presence or absence of **eaeA** sequences in 281 VTEC isolates from the feces of healthy cattle. There were 101 **eaeA**-positive isolates, which belonged to O groups 5, 26, 69, 80, 84, 98, 103, 111, 119, 145, 157 and 108 **eaeA**-negative isolates, which belonged to O groups 8, 22, 38, 113, 119, 116, 132, 153, 156 or untypable. All isolates were tested for hemolysis on horse blood agar and on washed sheep blood agar. PCR amplification was used to test for EHEC hemolysin, Ehly1, Ehly2 and alpha-hemolysin D sequences. Among **eaeA**-positive isolates 98% were positive for EHEC hemolysin sequences and were hemolytic on washed sheep red blood cell agar; the corresponding percentage for **eaeA** -negative isolates was 36%. Ehly1 and Ehly2 sequences were present in only 11 isolates (O groups 26, 84, 119 and 132). None of the **eaeA**-positive and 13 of the **eaeA**-negative isolates

(including all 11 isolates of O group 132) were positive for alpha-hemolysin D gene sequences by PCR and alpha-hemolysin production on horse blood agar. We conclude that since Ehly1, Ehly2 and alpha-hemolysin occur at low frequency among bovine VTEC and serotypes implicated in human disease they are unlikely to be significant virulence factors. In contrast, EHEC hemolysin was present in almost all **eaeA**-positive VTEC isolates and in approximately one-third the **eaeA**-negative ones; it may be both a virulence marker and virulence factor but further testing is required. The study identified isolates with all combinations of **eaeA** and EHEC hly, which may be useful for further testing.

L9 ANSWER 17 OF 24 MEDLINE DUPLICATE 9
ACCESSION NUMBER: 97101056 MEDLINE
DOCUMENT NUMBER: 97101056
TITLE: **Intimin** from enteropathogenic Escherichia coli restores murine virulence to a **Citrobacter rodentium eaeA** mutant: induction of an immunoglobulin A response to **intimin** and EspB.
AUTHOR: Frankel G; Phillips A D; Novakova M; Field H; Candy D C; Schauer D B; Douce G; Dougan G
CORPORATE SOURCE: Department of Biochemistry, Imperial College of Science, Technology and Medicine, London, United Kingdom.. g.frankel@ic.ac.uk
SOURCE: INFECTION AND IMMUNITY, (1996 Dec) 64 (12) 5315-25. Journal code: GO7. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199703
ENTRY WEEK: 19970302
AB The formation of attaching and effacing (A/E) lesions is central to the pathogenesis of enteropathogenic Escherichia coli (EPEC)-mediated disease in humans and **Citrobacter rodentium** (formerly C. freundii biotype 4280)-mediated transmissible colonic hyperplasia in mice. Closely related outer membrane proteins, known as **intimins**, are required for formation of the A/E lesion by both EPEC (Int(EPEC)) and C. rodentium (Int(CR)). A secreted protein, EspB (formally EaeB), is also necessary for A/E-lesion formation. Here we report that expression of a cloned Int(EPEC), encoded by plasmid pCVD438, restores murine virulence to an **intimin**-deficient mutant of C. rodentium DBS255. Replacement of Cys937 with Ala abolished the ability of the cloned EPEC **intimin** to complement the deletion mutation in DBS255. Ultrastructural examination of tissues from wild-type C. rodentium and DBS255(pCVD438)-infected mice revealed multiple A/E lesion on infected cells and loss of contact

between enterocytes and basement membrane. Histological investigation showed that although both wild-type C. rodentium and DBS255(pCVD438) colonized the descending colon and induced colonic hyperplasia in orally infected 21-day-old mice, the latter strain adhered to epithelial cells located deeper within crypts. Nonetheless, infection with the wild-type strain was consistently more virulent, as indicated by a higher mortality rate. All the surviving mice, challenged with either wild-type C. rodentium or DBS255(pCVD438), developed a mucosal immunoglobulin A response to **intimin** and EspB. These results show that C. rodentium infection provides a relevant, simple, and economic model to investigate the role of EPEC proteins in the formation of A/E lesions in vivo and in intestinal disease.

L9 ANSWER 18 OF 24 MEDLINE DUPLICATE 10
ACCESSION NUMBER: 96239042 MEDLINE
DOCUMENT NUMBER: 96239042
TITLE: Truncated **enterohemorrhagic** Escherichia **coli** (EHEC) O157:H7 **intimin** (**EaeA**) fusion proteins promote adherence of EHEC strains to HEp-2 cells.
AUTHOR: McKee M L; O'Brien A D
CORPORATE SOURCE: Department of Microbiology and Immunology, Uniformed Services University of the Health Sciences, F. Edward Hebert School of Medicine, Bethesda, Maryland 20814-4799, USA.
CONTRACT NUMBER: AI 21048-13 (NIAID)
SOURCE: INFECTION AND IMMUNITY, (1996 Jun) 64 (6) 2225-33. Journal code: GO7. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199610
AB **Intimin**, the product of the **eaeA** gene in **enterohemorrhagic** Escherichia **coli** O157:H7 (EHEC), is required for intimate adherence of these organisms to tissue culture cells and formation of the attaching and effacing lesion in the gnotobiotic pig. Because of the importance of **intimin** in the pathogenesis of EHEC O157:H7 infection in this animal model, we began a structure-function analysis of **EaeA**. For this purpose, we constructed amino-terminal fusions of the **intimin** protein with six histidine residues to form two independent fusions. The longer fusion, RIHisEae, contained 900 of the 935 predicted amino acids and included all but the extreme amino terminus. The second fusion, RVHdHisEae, consisted of the carboxyl two-thirds of the protein. Purified extracts of either construct enhanced binding of wild-type 86-24 to HEp-2 cells and conferred HEp-2 cell adherence on 86-24eaeDelta10, an **eaeA** deletion

mutant, and B2F1, an EHEC O91:1-121 **eaeA** mutant strain. When 86-24eaeDelta10 was transformed with either of the plasmids encoding the **intimin** fusion proteins, the transformant behaved like the wild-type parent strain and displayed localized adherence to HEp-2 cells, with positive fluorescent-actin staining. In addition, polyclonal antisera raised against RIHisEae reacted with both fusion constructs and recognized an outer membrane protein of the same mass as **intimin** (97 kDa) in EHEC and enteropathogenic E. **coli** but not E. **coli** K-12. The **intimin**-specific antisera also blocked adherence of EHEC to HEp-2 cells. Thus, **intimin** (i) is a 97-kDa outer membrane protein in EHEC that serves as a requisite adhesin for attachment of the bacteria to epithelial cells, even when the protein is truncated by one-third at its amino terminus and (ii) can be added exogenously to specifically facilitate HEp-2 cell adherence of EHEC but not E. **coli** K-12.

L9 ANSWER 19 OF 24 MEDLINE DUPLICATE 11
ACCESSION NUMBER: 97055784 MEDLINE
DOCUMENT NUMBER: 97055784
TITLE: Characterization of the **eaeA** gene from rabbit enteropathogenic Escherichia coli strain RDEC-1 and comparison to other **eaeA** genes from bacteria that cause attaching-effacing lesions.
AUTHOR: Agin T S; Cantey J R; Boedeker E C; Wolf M K
CORPORATE SOURCE: Walter Reed Army Institute of Research, Washington, DC 20307-5100, USA.
SOURCE: FEMS MICROBIOLOGY LETTERS, (1996 Nov 1) 144 (2-3) 249-58.
Journal code: FML. ISSN: 0378-1097.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U60002
ENTRY MONTH: 199704
ENTRY WEEK: 19970402
AB A number of enteric pathogens, including enteropathogenic (EPEC) and **enterohemorrhagic** (EHEC) Escherichia **coli**, **Hafnia alvei**, a strain of Citrobacter freundii, and rabbit EPEC strain RDEC-1 cause attaching-effacing (AE) lesions in the gut mucosa. These bacteria have a pathogenicity cassette (locus of enterocyte effacement or LEE) containing the **eaeA** gene. This gene encodes **intimin**, an outer membrane protein required for production of AE lesions. RDEC-1, a non-invasive enteropathogen in young rabbits, produces AE lesions morphologically indistinguishable from lesions caused by human AE bacterial strains. The RDEC-1 example of E. **coli** diarrhea in rabbits is an important model for studying the pathogenesis of AE bacteria in a

natural infection and for analyzing specific roles of the components of LEE. In order to better understand the role of **intimin** in the development of AE lesions, a portion of DNA within RDEC-1 LEE, containing the **eaeA** gene and an upstream open reading frame (ORF), was sequenced. The RDEC-1 **eaeA** gene shared 87%, 92%, and 93% DNA sequence identity and > 80% amino acid sequence identity with the **eaeA** genes of C. freundii biotype 4280, EHEC O157:H7, and EPEC O127:116, respectively. The carboxy-terminal 280 amino acid residues of **intimin** has 80%, 56%, and 54% identity with C. freundii, EHEC O157:H7, and EPEC O127:H6 **intimins**, respectively. The predicted protein encoded by the upstream ORF (156 amino acids) shares 95%, 97%, and 99% amino acid identity with predicted proteins from C. freundii. EHEC O157:H7, and EPEC O127:H6, respectively. The high degree of sequence homology of the ORF and the eueA gene of RDEC-1 with those of other AE bacteria suggests an evolutionary relationship of LEE and supports and facilitates the use of the RDEC-1 model for studying the role of LEE in pathogenesis.

L9 ANSWER 20 OF 24 MEDLINE DUPLICATE 12
ACCESSION NUMBER: 95369946 MEDLINE
DOCUMENT NUMBER: 95369946
TITLE: **Enterohemorrhagic** Escherichia **coli** O157:H7 requires **intimin** to colonize the gnotobiotic pig intestine and to adhere to HEp-2 cells.
AUTHOR: McKee M L; Melton-Celsa A R; Moxley R A; Francis D H; O'Brien A D
CORPORATE SOURCE: Department of Microbiology and Immunology, Uniformed Services University of the Health Sciences, F. Edward Hebert School of Medicine, Bethesda, Maryland 20814-4799, USA..
CONTRACT NUMBER: AI21048-12 (NIAID)
SOURCE: INFECTION AND IMMUNITY, (1995 Sep) 63 (9) 3739-44. Journal code: GO7. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199511
AB In a previous study, **enterohemorrhagic** Escherichia **coli** (EHEC) O157:H7 with a deletion and insertion in the **eaeA** gene encoding **intimin** was used to establish that **intimin** is required for the organism to attach to and efface microvilli in the piglet intestine (M. S. Donnenberg, S. Tzipori, M. L. McKee, A. D. O'Brien, J. Alroy, and J. B. Kaper, J. Clin. Invest. 92:1418-1424, 1993). However, in the same investigation, a role for **intimin** in EHEC adherence to HEp-2 cells could not be definitively demonstrated. To analyze the

basis for this discrepancy, we constructed an in-frame deletion of **eaeA** and compared the adherence capacity of this mutant with that of the wild-type strain in vitro and in vivo. We observed a direct correlation between the requisite for **intimin** in EHEC O157:H7 colonization of the gnotobiotic piglet intestine and adherence of the bacterium to HEp-2 cells. The in vitro-in vivo correlation lends credence to the use of the HEp-2 cell adherence model for further study of the **intimin** protein.

L9 ANSWER 21 OF 24 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 13
ACCESSION NUMBER: 1995:439486 BIOSIS
DOCUMENT NUMBER: PREV199598453786
TITLE: The role of the **eaeA** gene in diarrhea and neurological complications in a gnotobiotic piglet model of **enterohemorrhagic** Escherichia **coli** infection.
AUTHOR(S): Tzipori, Saul (1); Gunzer, Florian; Donnenberg, Michael S.; De Montigny, Lina; Kaper, Juames B.; Donohue-Rolfe, Arthur
CORPORATE SOURCE: (1) Div. Infectious Diseases, Tufts Univ. Sch. Vet. Med., 200 Westboro Rd., North Grafton, MA 01536 USA
SOURCE: Infection and Immunity, (1995) Vol. 63, No. 9, pp. 3621-3627.
ISSN: 0019-9567.
DOCUMENT TYPE: Article
LANGUAGE: English
AB We reported previously that mutation of the chromosomal gene **eaeA** from enterohemorragic Escherichia coli (EHEC) serotype 0157:H7 prevented bacterial attachment in vivo. Attachment was restored when the EHEC or enteropathogenic E. coli (EPEC) **eaeA** gene was introduced into the mutant on a plasmid. In this communication we have compared in gnotobiotic piglets the pathogenicities of wild-type 0157:H7 strain 86-24 and its **eaeA** mutant UMD619 with those of the two plasmid-complemented strains expressing **Intimin**-O157 (EHEC) and **Intimin**-O127 (EPEC). 86-24 colonized the surface and glandular epithelium of the large intestine an induced diarrhea, while UMD619 did not colonize any intestinal site and induced little or no diarrhea. Surprisingly, strain UMD619 expressing **Intimin**-O127 behaved in pigs more like EPEC than EHEC strains; it colonized the distal half of the small intestine and the surface of the large intestine, inducing serious diarrhea. In contrast, strain UMD619 expressing **Intimin** -O157 colonized the colon extremely poorly, inducing little or no diarrhea. While only the two strains causing extensive attachment-86-24 and UMD619 expressing **Intimin** -O127-induced diarrhea, neurological symptoms attributed to Shiga-like toxin II occurred equally in all four groups of animals. The intimate bacterial attachment and mucosal damage were not a

prerequisite for Shiga-like toxin II translocation from the gut lumen into the circulation. **Intimin**-O127 appears not only to facilitate intimate attachment to cells but also to influence the site of intestinal colonization and other characteristics of EPEC infection.

L9 ANSWER 22 OF 24 MEDLINE DUPLICATE 14
ACCESSION NUMBER: 95331475 MEDLINE
DOCUMENT NUMBER: 95331475
TITLE: Identification of **EaeA** protein in the outer membrane of attaching and effacing Escherichia coli O45 from pigs.
AUTHOR: Zhu C; Harel J; Dumas F; Fairbrother J M
CORPORATE SOURCE: Groupe de Recherche sur les Maladies Infectieuses du Porc, Universite de Montreal Faculte de Medecine Veterinaire, Saint-Hyacinthe, Quebec, Canada..
SOURCE: FEMS MICROBIOLOGY LETTERS, (1995 Jun 15) 129 (2-3) 237-42.
Journal code: FML. ISSN: 0378-1097.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199510
AB We have previously reported that the production of attaching and effacing lesions by Escherichia **coli** O45 isolates from pigs is associated with the **eaeA** (E. **coli** attaching and effacing) gene. In the present study, expression of the **EaeA** protein, the **eaeA** gene product, among swine O45 E. **coli** isolates was examined. The majority (20/22) of attaching and effacing positive, **eaeA**+ E. **coli** O45 isolates, but none of ten attaching and effacing negative, **eaeA**- or **eaeA**+ isolates, expressed a 97-kDa outer membrane protein as revealed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (**SDS-PAGE**) and Western blot analysis. Amino-terminal amino acid sequencing demonstrated a high homology between this 97-kDa protein of swine E. **coli** O45 and the **EaeA** protein ( **intimin**) of human enteropathogenic E. **coli** and **enterohemorrhagic** E. **coli**. In addition, a serological relationship between the **EaeA** proteins of swine O45, rabbit (RDEC-1) and human (E2348/69) attaching and effacing E. **coli** strains was observed. Our results indicate an association between expression of the **EaeA** protein and attaching and effacing activity among O45 E. **coli** isolates. The data also suggest an antigenic relatedness of the **EaeA** proteins of swine, rabbit, and human attaching and effacing E. **coli**.

L9 ANSWER 23 OF 24 BIOSIS COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER: 1995:290275 BIOSIS
DOCUMENT NUMBER: PREV199598304575
TITLE: Characteristics of an **Enterohemorrhagic** E. **coli eaeA** Mutant and Adherence Properties of Truncated **Intimin** (Eae) Protein Fusions.
AUTHOR(S): McKee, Marian L.; O'Brien, Alison D.
CORPORATE SOURCE: Uniformed Services Univ. Health Sci., Bethesda, MD USA
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (1995) Vol. 95, No. 0, pp. 166.
Meeting Info.: 95th General Meeting of the American Society for Microbiology Washington, D.C., USA May 21-25, 1995
ISSN: 1060-2011.
DOCUMENT TYPE: Conference
LANGUAGE: English

L9 ANSWER 24 OF 24 MEDLINE DUPLICATE 15
ACCESSION NUMBER: 94011289 MEDLINE
DOCUMENT NUMBER: 94011289
TITLE: Expression and characterization of the **eaeA** gene product of Escherichia coli serotype O157:H7.
AUTHOR: Louie M; de Azavedo J C; Handelsman M Y; Clark C G; Ally B; Dytoc M; Sherman P; Brunton J
CORPORATE SOURCE: Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada.
SOURCE: INFECTION AND IMMUNITY, (1993 Oct) 61 (10) 4085-92.
Journal code: GO7. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199401
AB In enteropathogenic Escherichia **coli**, the **eaeA** gene produces a 94-kDa outer membrane protein called **intimin** which has been shown to be necessary but not sufficient to produce the attaching-and-effacing lesion. The purpose of this study was to characterize the **intimin** specified by the **eaeA** allele of the **enterohemorrhagic** E. **coli** (EHEC) serotype O157:H7 strain CL8 and to determine its role in adherence. The carboxyl-terminal 266 amino acids of the CL8 **intimin** were expressed as a protein fusion with glutathione S-transferase, which was used to raise antiserum in rabbits. The antiserum reacted in Western immunoblots with a 97-kDa outer membrane protein of EHEC strains of serogroups O5, O26, O111, and O157 and enteropathogenic E. **coli** strains of serogroups O55 and O127. Surface

labelling of CL8 with 125I showed that **intimin** was surface exposed. An **eaeA** insertional inactivation mutant of CL8 was produced and was designated CL8-KO1. Total adherence of CL8-KO1 to HEp-2 cells was not significantly different from that of CL8, but CL8-KO1 gave a negative result in the fluorescent actin staining test. The **eaeA** gene expressed alone in E. **coli** HB101 also gave a negative fluorescent actin staining test result. The **eaeA** gene of CL8 was able to complement the **eaeA** deletion mutation in CVD206. We conclude that the product of the EHEC **eaeA** gene is a 97-kDa surface-exposed protein and propose that it be designated intiminO157. Sherman et al. described a 94-kDa outer membrane protein which played an important role in adherence of E. **coli** O157:H7 (Infect. Immun. 59:890-899, 1991). Western immunoblotting and indirect fluorescent antibody studies showed that the protein described by Sherman et al. is not **intimin**.

FILE 'CAPLUS' ENTERED AT 15:29:50 ON 26 JUL 2000
L10 28 S L3 AND EHEC
L11 14 S L10 AND (L1 OR INTIMIN OR SDSPAGE OR SDS PAGE)
L12 1 S L11 NOT L7

L12 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1997:459898 CAPLUS
DOCUMENT NUMBER: 127:120504
TITLE: Host exposure to **intimin** (**EaeA** ) prior to infection with an attaching/effacing rabbit enteropathogen
AUTHOR(S): Agin, Tonia S.; Noel, James M.; Mcqueen, Charles E.; Boedeker, Edgar C.; Wolf, Marcia K.
CORPORATE SOURCE: Walter Reed Army Institute of Research, Washington, DC, USA
SOURCE: Cytokines, Cholera Gut, [Pap. Jt. Meet. U. S.-Jpn. Coop. Med. Sci. Program Panels Malnutr. Cholera] (1997), Meeting Date 1995, 315-320. Editor(s): Keusch, Gerald T.; Kawakami, Masanobu. IOS Press: Amsterdam, Neth. CODEN: 64SIAE
DOCUMENT TYPE: Conference
LANGUAGE: English
AB Bacteria require the **intimin** (**EaeA**) protein to form attaching and effacing (A/E) lesions, characteristic of EPEC and **EHEC** infections in humans and RDEC-1 infections in rabbits, on intestinal epithelia. We retrospectively analyzed rabbit sera for the presence of anti-**intimin** IgG prior to infection with RDEC-1 or a deriv. of RDEC-1 that expresses SLT-1, to det. if there was a correlation between prior exposure to **intimin** and protection from disease. Five rabbits, three challenged with RDEC-1 and two challenged with RDEC-H19A, had

pre-existing anti-**intimin** IgG prior to challenge and all five animals showed good wt. gain post challenge. Thirty-two rabbits lacked pre-existing anti-**intimin** IgG and showed varing wt. gain post challenge. These results suggest that the rabbits with prior exposure to **intimin** were unlikely to be in the group exhibiting symptoms of RDEC-1 infection.

(FILE 'CAPLUS, MEDLINE, BIOSIS, EMBASE, WPIDS, JAPIO, JICST-EPLUS, TOXLIT, TOXLINE, PHIC, PHIN' ENTERED AT 15:32:27 ON 26 JUL 2000)

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L15        1032 S FINLAY B?/AU
L16         331 S KENNY B?/AU
L17          47 S (DEVINNEY R? OR DE VINNEY R?)/AU
L18        3870 S STEIN M?/AU
L19           8 S L15 AND L16 AND L17 AND L18
L20          93 S L15 AND (L16 OR L17 OR L18)
L21          29 S L16 AND (L17 OR L18)
L22          12 S L17 AND L18
L23        5146 S L15 OR L16 OR L17 OR L18
L24          49 S (L20 OR L21 OR L23) AND L3
L25          50 S L19 OR L22 OR L24
L26          19 DUP REM L25 (31 DUPLICATES REMOVED)
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- Author(s)

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=> d 1-19 ibib abs; fil hom

L26  ANSWER 1 OF 19  CAPLUS  COPYRIGHT 2000 ACS        DUPLICATE 1
ACCESSION NUMBER:         2000:438489  CAPLUS
TITLE:                    Mechanical fractionation reveals structural
                          requirements for enteropathogenic Escherichia
                          coli Tir insertion into host membranes
AUTHOR(S):                Gauthier, Annick; De Grado, Myriam; Finlay,
                          B. Brett
CORPORATE SOURCE:         Department of Biochemistry and Molecular Biology
                          and Biotechnology Laboratory, University of
                          British Columbia, Vancouver, BC, V6T 1Z3, Can.
SOURCE:                   Infect. Immun. (2000), 68(7), 4344-4348
                          CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER:                American Society for Microbiology
DOCUMENT TYPE:            Journal
LANGUAGE:                 English
```

AB Enteropathogenic Escherichia coli (EPEC) inserts its receptor for intimate adherence (**Tir**) into host cell membranes by using a type III secretion system. Detergents are frequently used to fractionate infected host cells to investigate bacterial protein delivery into mammalian cells. In this study, we found that the Triton X-100-sol. membrane fraction from EPEC-infected HeLa cells was contaminated with bacterial proteins. We therefore applied a mech. method of cell lysis and ultracentrifugation to fractionate infected HeLa cells to investigate the biol. and biochem. of **Tir** delivery and translocation. This method demonstrates

that the translocation of **Tir** into the host cell membrane requires its transmembrane domains, but not tyrosine phosphorylation or binding to **Tir**'s ligand, intimin.

REFERENCE COUNT: 31

REFERENCE(S):
(1) Abe, A; Mol Microbiol 1999, V33, P1162 CAPLUS
(2) Bauer, M; Trends Cell Biol 2000, V10, P25 CAPLUS
(3) Collazo, C; Mol Microbiol 1997, V24, P747 CAPLUS
(4) DeVinney, R; Cell Mol Life Sci 1999, V55, P961 CAPLUS
(5) DeVinney, R; Infect Immun 1999, V67, P2389 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L26 ANSWER 2 OF 19 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 2000:421954 CAPLUS
TITLE: Enteropathogenic E. coli **translocated intimin** receptor, **Tir**, interacts directly with .alpha.-actinin
AUTHOR(S): Goosney, Danika L.; **DeVinney, Rebekah**; Pfuetzner, Richard A.; Frey, Elizabeth A.; Strynadka, Natalie C.; **Finlay, B. Brett**
CORPORATE SOURCE: Biotechnology Laboratory, The Department of Microbiology and Immunology, University of British Columbia, Vancouver, BC, V6T 1Z3, Can.
SOURCE: Curr. Biol. (2000), 10(12), 735-738
CODEN: CUBLE2; ISSN: 0960-9822
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Enteropathogenic Escherichia coli (EPEC) triggers a dramatic rearrangement of the host epithelial cell actin cytoskeleton to form an attaching and effacing lesion, or pedestal. The pathogen remains attached extracellularly to the host cell through the pedestal for the duration of the infection. At the tip of the pedestal is a bacterial protein, **Tir**, which is secreted from the bacterium into the host cell plasma membrane, where it functions as the receptor for an EPEC outer membrane protein, intimin [1]. Delivery of **Tir** to the host cell results in its tyrosine phosphorylation, followed by **Tir**-intimin binding. **Tir** is believed to anchor EPEC firmly to the host cell, although its direct linkage to the cytoskeleton is unknown. Here, we show that **Tir** directly binds the cytoskeletal protein .alpha.-actinin. .alpha.-Actinin is recruited to the pedestal in a **Tir**-dependent manner and colocalizes with **Tir** in infected host cells. Binding is mediated through the amino terminus of **Tir**. Recruitment of .alpha.-actinin occurs

independently of **Tir** tyrosine phosphorylation. Recruitment of actin, VASP, and N-WASP, however, is abolished in the absence of this tyrosine phosphorylation. These results suggest that **Tir** plays at least three roles in the host cell during infection: binding intimin on EPEC; mediating a stable anchor with .alpha.-actinin through its amino terminus in a phosphotyrosine-independent manner; and recruiting addnl. cytoskeletal proteins at the carboxyl terminus in a phosphotyrosine-dependent manner. These findings demonstrate the first known direct linkage between extracellular EPEC, through the transmembrane protein **Tir**, to the host cell actin cytoskeleton via .alpha.-actinin.

REFERENCE COUNT: 14

REFERENCE(S):
(1) Abe, A; Mol Microbiol 1999, V33, P1162 CAPLUS
(2) deGrado, M; Cellular Microbiol 1999, V1, P7 CAPLUS
(3) Dramsi, S; Annu Rev Cell Dev Biol 1998, V14, P137 CAPLUS
(4) Frankel, G; Infect Immun 1995, V63, P4323 CAPLUS
(5) Frankel, G; J Biol Chem 1996, V271, P20359 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L26 ANSWER 3 OF 19 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 2

ACCESSION NUMBER: 2000:240248 CAPLUS
DOCUMENT NUMBER: 133:15368

TITLE: Enteropathogenic Escherichia coli (EPEC) attachment to epithelial cells: exploiting the host cell cytoskeleton from the outside

AUTHOR(S): Celli, Jean; Deng, Wanyin; **Finlay, B. Brett**
CORPORATE SOURCE: Biotechnology Laboratory, University of British Columbia, Vancouver, BC, V6T 1Z3, Can.

SOURCE: Cell. Microbiol. (2000), 2(1), 1-9
CODEN: CEMIF5; ISSN: 1462-5814
PUBLISHER: Blackwell Science Ltd.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

AB A review, with 54 refs. Enteropathogenic Escherichia coli (EPEC), a leading cause of human infantile diarrhea, is the prototype for a family of intestinal bacterial pathogens that induce attaching and effacing (A/E) lesions on host cells. A/E lesions are characterized by localized effacement of the brush border of enterocytes, intimate bacterial attachment and pedestal formation beneath the adherent bacteria. As a result of some recent breakthrough discoveries, EPEC has now emerged as a fascinating paradigm for the study of host-pathogen interactions and cytoskeletal rearrangements that

occur at the host cell membrane. EPEC uses a type III secretion machinery to attach to epithelial cells, translocating its own receptor for intimate attachment, **Tir**, into the host cell, which then binds to intimin on the bacterial surface. Studies of EPEC-induced cytoskeletal rearrangements have begun to provide clues as to the mechanisms used by this pathogen to subvert the host cell cytoskeleton and signaling pathways. These findings have unraveled new ways by which pathogenic bacteria exploit host processes from the cell surface and have shed new light on how EPEC might cause diarrhea.

REFERENCE COUNT: 54

REFERENCE(S):
(1) Abe, A; J Exp Med 1998, V188, P1907 CAPLUS
(2) Abe, A; Mol Microbiol 1999, V33, P1162 CAPLUS
(3) Adam, T; J Cell Biol 1995, V129, P367 CAPLUS
(4) Bain, C; Infect Immun 1998, V66, P3900 CAPLUS
(5) Baldwin, T; Infect Immun 1991, V59, P1599 CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L26 ANSWER 4 OF 19 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 3

ACCESSION NUMBER: 1999:577042 CAPLUS
DOCUMENT NUMBER: 131:194270
TITLE: Methods for assaying type III secretion inhibitors
INVENTOR(S): **Finlay, Brett B.; Kenny, Brendan; Stein, Marcus**
PATENT ASSIGNEE(S): University of British Columbia, Can.
SOURCE: PCT Int. Appl., 52 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 9945136 | A1 | 19990910 | WO 1999-CA183 | 19990305 |
| W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | | |
| AU 9932431 | A1 | 19990920 | AU 1999-32431 | 19990305 |

Searcher : Shears 308-4994

PRIORITY APPLN. INFO.: US 1998-76980 19980305
WO 1999-CA183 19990305

AB A method is provided for identifying compds. that specifically inhibit type III secretion systems that are used by several Gram-neg. animal and plant pathogens to secrete virulence factors that are crit. in causing disease. The compds. identified by this method are used as new antibacterial therapeutics. Specific inhibitors of the enteropathogenic Escherichia coli (EPEC) type III secretion system, that block EPEC signaling in host cells are identified by the use of specific mol. tools that have been developed with EPEC, including specific antibodies to secreted proteins and genetic fusions of epitope tags to genes encoding these secreted products. Promising compds. identified with the EPEC system are tested for their ability to inhibit type III secretion systems in other medically important pathogens.

REFERENCE COUNT: 2
REFERENCE(S): (1) Brett, F; WO 9740063 A 1997
(2) Holden, D; WO 9617951 A 1996

L26 ANSWER 5 OF 19 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 4
ACCESSION NUMBER: 1999:326051 CAPLUS
DOCUMENT NUMBER: 130:333761
TITLE: Pathogenic Escherichia coli intimin receptor **Tir** and gene **tir** and methods for detecting gene **tir** or **Tir** protein and for drug screening
INVENTOR(S): **Finlay, B. Brett; Kenny, Brendan; Devinney, Rebekah; Stein, Marcus**
PATENT ASSIGNEE(S): University of British Columbia, Can.
SOURCE: PCT Int. Appl., 91 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 9924576 | A1 | 19990520 | WO 1998-CA1042 | 19981110 |

W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AU 9911373 A1 19990531 AU 1999-11373 19981110
PRIORITY APPLN. INFO.: US 1997-65130 19971112
WO 1998-CA1042 19981110

AB A polypeptide, called **Tir** (for **translocated intimin** receptor), which is secreted by attaching and effacing pathogens, such as the enteropathogenic (EPEC) and enterohemorrhagic (EHEC) E. coli is disclosed. These bacterial pathogens insert their own receptors into mammalian cell surfaces, to which the bacterial pathogen then adheres to trigger addnl. host signaling events and actin nucleation. Diagnosis of disease caused by pathogenic E. coli can be performed by the use of antibodies which bind to **Tir** to detect the protein or the use of nucleic acid probes for detection of nucleic acids encoding **Tir** polypeptide. Isolated nucleic acid sequences encoding **Tir** polypeptide, **Tir** peptides, a recombinant method for producing recombinant **Tir**, antibodies which bind to **Tir**, and a kit for the detection of **Tir** -producing E. coli are provided. A method of immunizing a host with **Tir** to induce a protective immune response to **Tir** or a second polypeptide of interest is also provided. A method for screening for compds. which interfere with the binding of bacterial pathogens to their receptors is further provided. Thus, protein Hp90, previously believed to be a host membrane protein, has been identified as an EHEC- or EPEC-secreted protein which acts as an intimin receptor. Proteins encoded by the espA and espB genes were necessary for delivery of **Tir** to the host membrane.

REFERENCE COUNT: 6
REFERENCE(S): (1) Deibel, C; Molecular Microbiology 1998, V28(3), P463 CAPLUS
(2) Kenny, B; Cell 1997, V91, P511 CAPLUS
(3) Kenny, B; Infection and Immunity 1997, V65(7), P2528 CAPLUS
(4) Paton, A; Database EMBL - EMPRO 1998
(5) Paton, A; Infection and Immunity 1998, V66(11), P5580 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L26 ANSWER 6 OF 19 TOXLIT
ACCESSION NUMBER: 1999:23044 TOXLIT
DOCUMENT NUMBER: CA-130-333761K
TITLE: Pathogenic Escherichia coli intimin receptor **Tir** and gene **tir** and methods for detecting gene **tir** or **Tir** protein and for drug screening.
AUTHOR: **Finlay BB; Kenny B; Devinney R; Stein M**
SOURCE: (1999). PCT Int. Appl. PATENT NO. 9924576 05/20/1999 (University of British Columbia). CODEN: PIXXD2.

PUB. COUNTRY: CANADA
DOCUMENT TYPE: Patent
FILE SEGMENT: CA
LANGUAGE: English
OTHER SOURCE: CA 130:333761
ENTRY MONTH: 199906

AB A polypeptide, called **Tir** (for **translocated intimin** receptor), which is secreted by attaching and effacing pathogens, such as the enteropathogenic (EPEC) and enterohemorrhagic (EHEC) E. coli is disclosed. These bacterial pathogens insert their own receptors into mammalian cell surfaces, to which the bacterial pathogen then adheres to trigger addnl. host signaling events and actin nucleation. Diagnosis of disease caused by pathogenic E. coli can be performed by the use of antibodies which bind to **Tir** to detect the protein or the use of nucleic acid probes for detection of nucleic acids encoding **Tir** polypeptide. Isolated nucleic acid sequences encoding **Tir** polypeptide, **Tir** peptides, a recombinant method for producing recombinant **Tir**, antibodies which bind to **Tir**, and a kit for the detection of **Tir** -producing E. coli are provided. A method of immunizing a host with **Tir** to induce a protective immune response to **Tir** or a second polypeptide of interest is also provided. A method for screening for compds. which interfere with the binding of bacterial pathogens to their receptors is further provided. Thus, protein Hp90, previously believed to be a host membrane protein, has been identified as an EHEC- or EPEC-secreted protein which acts as an intimin receptor. Proteins encoded by the espA and espB genes were necessary for delivery of **Tir** to the host membrane.

L26 ANSWER 7 OF 19 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 5
ACCESSION NUMBER: 1999:651530 CAPLUS
DOCUMENT NUMBER: 131:348882
TITLE: Type III secretion-dependent hemolytic activity of enteropathogenic Escherichia coli
AUTHOR(S): Warawa, Jonathan; **Finlay, B. Brett**; **Kenny, Brendan**
CORPORATE SOURCE: Department of Pathology and Microbiology, School of Medical Sciences, Bristol, BS8 1TD, UK
SOURCE: Infect. Immun. (1999), 67(10), 5538-5540
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Enteropathogenic Escherichia coli (EPEC) was found to exhibit a type III secretion-dependent, contact-mediated, hemolytic activity requiring the EspA, EspB, and EspD secreted proteins. EspB and EspD display homol. to pore-forming mols. Our data suggest a mechanism to explain the requirement for all three Esp proteins in the

transfer of EPEC proteins, such as **Tir**, into target cells.

REFERENCE COUNT: 27

REFERENCE(S):
(2) Deibel, C; Mol Microbiol 1998, V28, P463 CAPLUS
(3) Donnenberg, M; Gene 1997, V184, P107 CAPLUS
(4) Donnenberg, M; Infect Immun 1990, V58, P1565 CAPLUS
(5) Elliott, S; Mol Microbiol 1998, V28, P1 CAPLUS
(7) High, N; EMBO J 1992, V11, P1991 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L26 ANSWER 8 OF 19 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 6

ACCESSION NUMBER: 1999:291203 CAPLUS
DOCUMENT NUMBER: 131:85390
TITLE: Enterohemorrhagic Escherichia coli O157:H7 produces **Tir**, which is translocated to the host cell membrane but is not tyrosine phosphorylated
AUTHOR(S): **DeVinney, Rebekah**; **Stein, Markus**; Reinscheid, Dieter; Abe, Akio; Ruschkowski, Sharon; **Finlay, B. Brett**
CORPORATE SOURCE: Biotechnology Laboratory, University of British Columbia, Vancouver, BC, V6T 1ZA, Can.
SOURCE: Infect. Immun. (1999), 67(5), 2389-2398 CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Intimate attachment to the host cell leading to the formation of attaching and effacing (A/E) lesions is an essential feature of enterohemorrhagic Escherichia coli (EHEC) O157:H7 pathogenesis. In a related pathogen, enteropathogenic E. coli (EPEC), this activity is dependent upon translocation of the intimin receptor, **Tir**, which becomes tyrosine phosphorylated within the host cell membrane. In contrast, the accumulation of tyrosine-phosphorylated proteins beneath adherent EHEC bacteria does not occur, leading to questions about whether EHEC uses a **Tir**-based mechanism for adherence and A/E lesion formation. In this report, we demonstrate that EHEC produces a functional **Tir** that is inserted into host cell membranes, where it serves as an intimin receptor. However, unlike in EPEC, in EHEC **Tir** is not tyrosine phosphorylated yet plays a key role in both bacterial adherence to epithelial cells and pedestal formation. EHEC, but not EPEC, was unable to synthesize **Tir** in Luria-Bertani medium but was able to secrete **Tir** into M9 medium, suggesting that **Tir** synthesis and secretion may be regulated differently in these two pathogens. EHEC **Tir** and EPEC **Tir** both bind intimin and focus cytoskeletal rearrangements,

indicating that tyrosine phosphorylation is not needed for pedestal formation. EHEC and EPEC intimins are functionally interchangeable, but EHEC **Tir** shows a much greater affinity for EHEC intimin than for EPEC intimin. These findings highlight some of the differences and similarities between EHEC and EPEC virulence mechanisms, which can be exploited to further define the mol. basis of pedestal formation.

REFERENCE COUNT: 45

REFERENCE(S):
(1) Abe, A; Infect Immun 1997, V65, P3547 CAPLUS
(4) Bieber, D; Science 1998, V280, P2114 CAPLUS
(5) Brunder, W; V Mol:Microbiol 1997, V24, P767 CAPLUS
(6) Dean-Nystrom, E; Infect Immun 1998, V66, P4560 CAPLUS
(7) Deibel, C; Mol Microbiol 1998, V28, P463 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L26 ANSWER 9 OF 19 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 7

ACCESSION NUMBER: 1999:159076 CAPLUS
DOCUMENT NUMBER: 130:308856

TITLE: Phosphorylation of tyrosine 474 of the enteropathogenic Escherichia coli (EPEC) **Tir** receptor molecule is essential for actin nucleating activity and is preceded by additional host modifications

AUTHOR(S): **Kenny, Brendan**
CORPORATE SOURCE: Department of Pathology and Microbiology, School of Medical Sciences, University Walk, Bristol, BS8 1TD, UK

SOURCE: Mol. Microbiol. (1999), 31(4), 1229-1241
CODEN: MOMIEE; ISSN: 0950-382X
PUBLISHER: Blackwell Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The enteropathogenic Escherichia coli (EPEC) **Tir** protein becomes tyrosine phosphorylated in host cells and displays an increase in apparent mol. mass. The interaction of **Tir** with the EPEC outer membrane protein, intimin, triggers actin nucleation beneath the adherent bacteria. The enterohaemorrhagic E. coli 0157:H7 (EHEC) **Tir** mol. is not tyrosine phosphorylated. In this paper, **Tir** tyrosine phosphorylation is shown to be essential for actin nucleation activity, but not for the increase in apparent mol. mass obsd. in target cells. Tyrosine phosphorylation had no role in **Tir** mol. mass shift, indicating addnl. host modifications. Anal. of **Tir** intermediates indicates that tyrosine-independent modification functions to direct **Tir**'s correct insertion from the cytoplasm into the host membrane. Deletion anal.

identified **Tir** domains participating in translocation, assocn. with the host membrane, modification and antibody recognition. Intimin was found to bind a 55-amino-acid region (TIBA) within **Tir** that topol. and sequence anal. suggests is located in an extracellular loop. Homologous TIBA sequences exist in integrins, which also bind intimin. Collectively, this study provides definitive evidence for the importance of tyrosine phosphorylation for EPEC **Tir** function and reveals differences in the pathogenicity of EPEC and EHEC. The data also suggest a mechanism for **Tir** insertion into the host membrane, as well as providing clues to the mode of intimin-integrin interaction.

REFERENCE COUNT: 33
REFERENCE(S):
(1) Abe, A; Infect Immun 1997, V65, P3547 CAPLUS
(2) Adu-Bobie, J; J Clin Microbiol 1998, V36, P662 CAPLUS
(3) Anderson, D; Science 1997, V278, P1140 CAPLUS
(4) Beaulieu, J; J Cell Sci 1992, V102, P427 CAPLUS
(5) Clark, M; Infect Immun 1998, V66, P1237 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L26 ANSWER 10 OF 19 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 8
ACCESSION NUMBER: 1999:632013 CAPLUS
DOCUMENT NUMBER: 131:333662
TITLE: Enteropathogenic Escherichia coli **translocated intimin** receptor, **Tir**, requires a specific chaperone for stable secretion
AUTHOR(S): Abe, Akio; De Grado, Myriam; Pfuetzner, Richard A.; Sanchez-SanMartin, Claudia; **DeVinney, Rebekah**; Puente, Jose Luis; Strynadka, Natalie C. J.; **Finlay, B. Brett**
CORPORATE SOURCE: Biotechnology Laboratory, University of British Columbia, Vancouver, BC, V6T 1Z3, Can.
SOURCE: Mol. Microbiol. (1999), 33(6), 1162-1175
CODEN: MOMIEE; ISSN: 0950-382X
PUBLISHER: Blackwell Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Enteropathogenic Escherichia coli (EPEC) secretes several Esps (E. coli-secreted proteins) that are required for full virulence. Insertion of the bacterial protein **Tir** into the host epithelial cell membrane is facilitated by a type III secretion app., and at least EspA and EspB are required for **Tir** translocation. An EPEC outer membrane protein, intimin, interacts with **Tir** on the host membrane to establish intimate

attachment and formation of a pedestal-like structure. In this study, we identified a **Tir** chaperone, CesT, whose gene is located between **tir** and eae (which encodes intimin). A mutation in cesT abolished **Tir** secretion into culture supernatants and significantly decreased the amt. of **Tir** in the bacterial cytoplasm. In contrast, this mutation did not affect the secretion of the Esp proteins. The level of **tir** mRNA was not affected by the cesT mutation, indicating that CesT acts at the post-transcriptional level. The cesT mutant could not induce host cytoskeletal rearrangements, and displayed the same phenotype as the **tir** mutant. Gel overlay and GST pulldown assays demonstrated that CesT specifically interacts with **Tir**, but not with other Esp proteins. Furthermore, by using a series of **Tir** deletion derivs., we detd. that the CesT binding domain is located within the first 100 amino-terminal residues of **Tir**, and that the pool of **Tir** in the bacterial cytoplasm was greatly reduced when this domain was disrupted. Interestingly, this domain was not sufficient for **Tir** secretion, and at least the first 200 residues of **Tir** were required for efficient secretion. Gel filtration studies showed that **Tir**-CesT forms a large multimeric complex. Collectively, these results indicate that CesT is a **Tir** chaperone that may act as an anti-degrdn. factor by specifically binding to its amino-terminus, forming a multimeric stabilized complex.

REFERENCE COUNT: 57

REFERENCE(S):
(1) Abe, A; J Exp Med 1998, V188, P1907 CAPLUS
(2) An, H; FEMS Microbiol Lett 1997, V148, P239 CAPLUS
(3) Anderson, D; Science 1997, V278, P1140 CAPLUS
(4) Beaudry, M; J Clin Microbiol 1996, V34, P144 CAPLUS
(6) Cheng, L; Mol Microbiol 1997, V24, P757 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L26 ANSWER 11 OF 19 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 9

ACCESSION NUMBER: 1999:422677 CAPLUS

TITLE: Enteropathogenic Escherichia coli. A pathogen that inserts its own receptor into host cells

AUTHOR(S): **De Vinney, R.**; Gauthier, A.; Abe, A.; **Finlay, B. B.**

CORPORATE SOURCE: Biotechnology Laboratory, Univ. British Columbia, Vancouver, BC, V6T 1Z4, Can.

SOURCE: Cell. Mol. Life Sci. (1999), 55(6/7), 961-976
CODEN: CMLSFI; ISSN: 1420-682X

PUBLISHER: Birkhaeuser Verlag

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Enteropathogenic Escherichia coli (EPEC) is a major cause of infant diarrhea, killing hundreds of thousands of children per yr worldwide. Intimate attachment to the host cell leading to the formation of actin-rich pedestals beneath the adhering bacteria is an essential feature of EPEC pathogenesis. EPEC attaches to host cells via the outer membrane adhesin, intimin. It was recently shown that EPEC inserts its own receptor for intimate adherence, **Tir** (**translocated intimin** receptor) into the host cell membrane. The focus of this review is on the discovery and characterization of this novel receptor, and our current understanding of its role in pedestal formation. Gram-neg. bacterial secretion systems, including type III secretion systems, are reviewed and discussed in the context of **Tir** delivery into the host cell membrane. The relationship and relevance of in vitro models compared to the actual in viva situation is essential to understanding disease. We have critically reviewed the use of animal models in studying EPEC infection. Elucidating the function of **Tir** will contribute to our understanding of how EPEC mediates disease.

REFERENCE COUNT: 122

REFERENCE(S):
(1) Abe, A; Infect Immun 1997, V65, P3547 CAPLUS
(3) Adams, L; Infect Immun 1997, V65, P5222 CAPLUS
(4) Allaoui, A; J Bacteriol 1994, V176, P4534 CAPLUS
(5) Allaoui, A; Mol Microbiol 1995, V18, P343 CAPLUS
(6) Anderson, D; Science 1997, V278, P1140 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L26 ANSWER 12 OF 19 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 10

ACCESSION NUMBER: 1999:83261 CAPLUS

DOCUMENT NUMBER: 130:236380

TITLE: Enteropathogenic Escherichia coli inhibits phagocytosis

AUTHOR(S): Goosney, Danika L.; Celli, Jean; **Kenny, Brendan**; **Finlay, B. Brett**

CORPORATE SOURCE: Biotechnology Laboratory and Departments of Microbiology & Immunology and of Biochemistry & Molecular Biology, University of British Columbia, Vancouver, BC, V6T 1Z3, Can.

SOURCE: Infect. Immun. (1999), 67(2), 490-495
CODEN: INFIBR; ISSN: 0019-9567

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Enteropathogenic Escherichia coli (EPEC) interacts with intestinal

epithelial cells, activating host signaling pathways leading to cytoskeletal rearrangements and ultimately diarrhea. Here it is shown that EPEC interacts with the macrophage-like cell line J774A.1 to inhibit phagocytosis by these cells. Antiphagocytic activity was also obsd. in cultured RAW macrophage-like cells upon EPEC infection. The EPEC antiphagocytic phenotype was dependent on the type III secretion pathway of EPEC and its secreted proteins, including EspA, EspB, and EspD. Intimin and **Tir** mutants displayed intermediate antiphagocytic activity, suggesting that intimate attachment mediated by intimin-**Tir** binding may also play a role in antiphagocytosis. Tyrosine dephosphorylation of several host proteins was obsd. following infection with secretion-competent EPEC but not with secretion-deficient mutants. Dephosphorylation was detectable 120 min after infection with EPEC, directly correlating with the onset of the antiphagocytic phenotype. Inhibition of protein tyrosine phosphatases by pervanadate treatment increased the no. of intracellular wild-type EPEC organisms to levels seen with secretion-deficient mutants, suggesting that dephosphorylation events are linked to the antiphagocytic phenotype. No tyrosine phosphatase activity was detected with the EPEC-secreted proteins, suggesting that EPEC induces antiphagocytosis via a different mechanism than Yersinia species. The present findings demonstrate a novel function for EPEC-secreted proteins in triggering macrophage protein tyrosine dephosphorylation and inhibition of phagocytosis.

REFERENCE COUNT: 31

REFERENCE(S):
(1) Abe, A; Infect Immun 1997, V65, P3547 CAPLUS
(2) Andersson, K; Mol Microbiol 1996, V20, P1057 CAPLUS
(3) Baldwin, T; Infect Immun 1991, V59, P1599 CAPLUS
(4) Bliska, J; Proc Natl Acad Sci USA 1991, V88, P1187 CAPLUS
(6) Donnenberg, M; Infect Immun 1990, V58, P1565 CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L26 ANSWER 13 OF 19 MEDLINE DUPLICATE 11

ACCESSION NUMBER: 1999158956 MEDLINE

DOCUMENT NUMBER: 99158956

TITLE: Enteropathogenic Escherichia coli: cellular harassment.

AUTHOR: **DeVinney R**; Knoechel D G; **Finlay B B**

CORPORATE SOURCE: Biotechnology Laboratory University of British Columbia Vancouver British Columbia V6T 1Z4 Canada.

SOURCE: Curr Opin Microbiol, (1999 Feb) 2 (1) 83-8. Ref: 47
Journal code: DAY. ISSN: 1369-5274.

PUB. COUNTRY: ENGLAND: United Kingdom

Searcher : Shears 308-4994

Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199906
ENTRY WEEK: 19990603
AB The mechanisms by which enteropathogenic Escherichia coli (EPEC) mediates diarrhea remain a mystery. Recently a number of interesting and at times surprising results have come from studying EPEC interactions with host cells. Identification and characterization of bacterial factors, including **Tir**, EspA, EspB and EspD, and host responses have expanded our grasp of the diverse effects of EPEC on host cells.

L26 ANSWER 14 OF 19 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 2000:298095 CAPLUS
TITLE: Identification of the intimin-binding domain of **Tir** of enteropathogenic Escherichia coli
AUTHOR(S): De Grado, Myriam; Abe, Akio; Gauthier, Annick; Steele-Mortimer, Olivia; **DeVinney, Rebekah**; **Finlay, B. Brett**
CORPORATE SOURCE: Biotechnology Laboratory, University of British Columbia, Vancouver, BC, V6T 1Z3, Can.
SOURCE: Cell. Microbiol. (1999), 1(1), 7-17
CODEN: CEMIF5; ISSN: 1462-5814
PUBLISHER: Blackwell Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Enteropathogenic Escherichia coli (EPEC) attaches intimately to mammalian cells via a bacterial outer membrane adhesion mol., intimin, and its receptor in the host cell membrane, **Tir**. **Tir** is a bacterial protein translocated into the host cell membrane and tyrosine phosphorylated after insertion. **Tir** -intimin binding induces organized actin polymn. beneath the adherent bacteria, resulting in the formation of pedestal-like structures. A series of **Tir** deletion derivs. were constructed to analyze which **Tir** domains are involved in intimin binding. We have localized the intimin-binding domain (IBD) of **Tir** using a yeast two-hybrid system and a gel-overlay approach to a region of 109 amino acids that is predicted to be exposed on the surface of the plasma membrane. A truncated **Tir** protein lacking this domain was translocated to the host cell membrane and tyrosine phosphorylated, but failed to bind intimin or to induce either actin polymn. or **Tir** accumulation beneath the bacteria. These results indicate that only a small region of **Tir** is needed to bind intimin and support the predicted topol. for **Tir**, with both N- and C-terminal regions in the mammalian cell cytosol. They also confirm

that **Tir**-intimin interactions are needed for cytoskeletal organization. We have also identified N-terminal regions involved in **Tir** stability and **Tir** secretion to the media.

REFERENCE COUNT: 27
REFERENCE(S):
(1) DeVinney, R; Infect Immun 1999, V67, P2389 CAPLUS
(3) Donnenberg, M; Infect Immun 1991, V59, P4310 CAPLUS
(4) Donnenberg, M; J Bacteriol 1993, V175, P4670 CAPLUS
(5) Donnenberg, M; J Clin Invest 1993, V92, P1412 CAPLUS
(6) Elliott, S; Mol Microbiol 1998, V28, P1 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L26 ANSWER 15 OF 19 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 1998187833 EMBASE
TITLE: EPEC delivers the goods.
AUTHOR: Kaper J.B.; **Finlay B.B.**; **DeVinney R.**; **Kenny B.**; **Stein M.**
CORPORATE SOURCE: J.B. Kaper, Center for Vaccine Development, University of Maryland, School of Medicine, 685 West Baltimore St, Baltimore, MD 21201, United States. jkaper@umaryland.edu
SOURCE: Trends in Microbiology, (1998) 6/5 (169-172). Refs: 20
ISSN: 0966-842X CODEN: TRMIEA
PUBLISHER IDENT.: S 0966-842X(98)01266-9
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Note
FILE SEGMENT: 004 Microbiology
029 Clinical Biochemistry
048 Gastroenterology
LANGUAGE: English

L26 ANSWER 16 OF 19 MEDLINE
ACCESSION NUMBER: 1999227887 MEDLINE
DOCUMENT NUMBER: 99227887
TITLE: Enteropathogenic E. coli interactions with host cells.
AUTHOR: **Finlay B B**; Abe A
CORPORATE SOURCE: Biotechnology Laboratory, University of British Columbia, Vancouver, Canada.
SOURCE: JAPANESE JOURNAL OF MEDICAL SCIENCE AND BIOLOGY, (1998) 51 Suppl S91-100. Ref: 17
Journal code: KLZ. ISSN: 0021-5112.
PUB. COUNTRY: Japan
Journal; Article; (JOURNAL ARTICLE)
Searcher : Shears 308-4994

General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199909
ENTRY WEEK: 19990902

AB Enteropathogenic E. coli (EPEC) interacts with intestinal epithelial cells, causing diarrhea and associated diseases. This pathogen binds to epithelial cells using sophisticated mechanisms that exploit existing epithelial signal transduction pathways and host cytoskeletal components, ultimately resulting in the bacterium resting upon a pedestal on host cell surfaces. Recent data indicates that similar mechanisms occur in vivo. EPEC interactions with host cells illustrate several principles of pathogenesis that are used by bacteria that interact with mammalian host cells.

L26 ANSWER 17 OF 19 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 12
ACCESSION NUMBER: 1997:755652 CAPLUS
DOCUMENT NUMBER: 128:72707
TITLE: Enteropathogenic E. coli (EPEC) transfers its receptor for intimate adherence into mammalian cells
AUTHOR(S): **Kenny, Brendan; DeVinney, Rebekah; Stein, Markus;** Reinscheid, Dieter J.; Frey, Elizabeth A.; **Finlay, B. Brett**
CORPORATE SOURCE: Biotechnol. Lab., Dep. Biochem. Mol. Biol., Dep. Microbiol. Immunology, Univ. British Columbia, Vancouver, BC, V6T 1Z3, Can.
SOURCE: Cell (Cambridge, Mass.) (1997), 91(4), 511-520
CODEN: CELLB5; ISSN: 0092-8674
PUBLISHER: Cell Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Enteropathogenic Escherichia coli (EPEC) belongs to a group of bacterial pathogens that induce epithelial cell actin rearrangements resulting in pedestal formation beneath adherent bacteria. This requires the secretion of specific virulence proteins needed for signal transduction and intimate adherence. EPEC interaction induces tyrosine phosphorylation of a protein in the host membrane, Hp90, which is the receptor for the EPEC outer membrane protein, intimin. Hp90-intimin interaction is essential for intimate attachment and pedestal formation. Here, we demonstrate that Hp90 is actually a bacterial protein (**Tir**). Thus, this bacterial pathogen inserts its own receptor into mammalian cell surfaces, to which it then adheres to trigger addnl. host signaling events and actin nucleation. It is also tyrosine-phosphorylated upon transfer into the host cell.

L26 ANSWER 18 OF 19 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1996:381158 CAPLUS
DOCUMENT NUMBER: 125:53467
TITLE: A pathogenic bacterium triggers epithelial signals to form a functional bacterial receptor that mediates actin pseudopod formation
AUTHOR(S): Rosenshine, Ilan; Ruschkowski, Sharon; **Stein, Markus**; Reinscheid, Dieter J.; Mills, Scott D.; **Finlay, B. Brett**
CORPORATE SOURCE: Department Biotechnology and Molecular Genetics, Hebrew University, Jerusalem, 12272, Israel
SOURCE: EMBO J. (1996), 15(11), 2613-2624
CODEN: EMJODG; ISSN: 0261-4189
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Enteropathogenic Escherichia coli (EPEC) belongs to a group of bacterial pathogens that induce actin accumulation beneath adherent bacteria. We found that EPEC adherence to epithelial cells mediates the formation of finger-like pseudopods (up to 10 .mu.m) beneath bacteria. These actin-rich structures also contain tyrosine phosphorylated host proteins concd. at the pseudopod tip beneath adherent EPEC. Intimate bacterial adherence (and pseudopod formation) occurred only after prior bacterial induction of tyrosine phosphorylation of an epithelial membrane protein, Hp90, which then assocs. directly with an EPEC adhesin, intimin. These interactions lead to cytoskeletal nucleation and pseudopod formation. This is the first example of a bacterial pathogen that triggers signals in epithelial cells which activates receptor binding activity to a specific bacterial ligand and subsequent cytoskeletal rearrangement.

L26 ANSWER 19 OF 19 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 13
ACCESSION NUMBER: 1996:286497 CAPLUS
DOCUMENT NUMBER: 125:2537
TITLE: EspA, a protein secreted by enteropathogenic Escherichia coli, is required to induce signals in epithelial cells
AUTHOR(S): **Kenny, Brendan**; Lai, Li-Ching; **Finlay, B. Brett**; Donnenberg, Michael S.
CORPORATE SOURCE: Dep of Biochemistry and Molecular Biology, Univ. of British Columbia, Vancouver, BC, V6T 1Z3, Can.
SOURCE: Mol. Microbiol. (1996), 20(2), 313-323
CODEN: MOMIEE; ISSN: 0950-382X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Enteropathogenic Escherichia coli (EPEC) is a leading cause of infant diarrhea. EPEC mediates several effects on host epithelial cells, including activation of signal-transduction pathways, cytoskeletal rearrangement along with pedestal and

attaching/effacing lesion formation. It has been previously shown that the EPEC eaeB (espB) gene encodes a secreted protein required for signal transduction and adherence, while **eaeA** encodes intimin, an EPEC membrane protein that mediates intimate adherence and contributes to focusing of cytoskeletal proteins beneath bacteria. DNA-sequence anal. of a region between **eaeA** and eaeB identified a predicted open reading frame (espA) that matched the amino-terminal sequence of a 25 kDa EPEC secreted protein. A mutant with a non-polar insertion in espA does not secrete this protein, activate epithelial cell signal transduction or cause cytoskeletal rearrangement. These phenotypes were complemented by a cloned espA gene. The espA mutant is also defective for invasion. It is concluded that espA encodes an EPEC secreted protein that is necessary for activating epithelial signal transduction, intimate contact, and formation of attaching and effacing lesions, processes which are central to pathogenesis.

FILE 'CAPLUS' ENTERED AT 15:53:13 ON 26 JUL 2000

L1 16 SEA FILE=REGISTRY ABB=ON PLU=ON INTIMIN ?/CN

L2 2 SEA FILE=REGISTRY ABB=ON PLU=ON TRANSLOCATED INTIMIN RECEPTOR?/CN

L3 616 SEA FILE=CAPLUS ABB=ON PLU=ON L2 OR TIR OR TRANSLOCAT? INTIMIN OR EAEA OR EAE A

L27 92 SEA FILE=CAPLUS ABB=ON PLU=ON L3 AND (PATHOGEN OR ENTEROPATHOG?(S)COLI)

L30 54 SEA FILE=CAPLUS ABB=ON PLU=ON L27 AND (L1 OR INTIMIN)

L31 1 S L30 AND (SDSPAGE OR ((NA OR SODIUM)(W)DODECYL OR SDS)(5W)(PAGE OR ELECTROPHOR?))

L31 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1995:641439 CAPLUS

DOCUMENT NUMBER: 123:51827

TITLE: Identification of **EaeA** protein in the outer membrane of attaching and effacing Escherichia coli O45 from pigs

AUTHOR(S): Zhu, Chengru; Harel, Josee; Dumas, France; Fairbrother, John M.

CORPORATE SOURCE: Groupe de Recherche sur les Maladies Infectieuses du Porc, Universite de Montreal, Faculte de Medecine Veterinaire, C.P. 5000, Saint-Hyacinthe, Can.

SOURCE: FEMS Microbiol. Lett. (1995), 129(2-3), 237-42
CODEN: FMLED7; ISSN: 0378-1097

DOCUMENT TYPE: Journal

LANGUAGE: English

AB We have previously reported that the prodn. of attaching and effacing lesions by Escherichia coli O45 isolates from pigs is assocd. with the **eaeA** (E. coli attaching and effacing)

gene. In the present study, expression of the **EaeA** protein, the **eaeA** gene product, among swine O45 E. coli isolates was examd. The majority (20/22) of attaching and effacing pos., **eaeA**+ E. coli O45 isolates, but none of ten attaching and effacing neg., **eaeA**- or **eaeA**+ isolates, expressed a 97-kDa outer membrane protein as revealed by **sodium dodecyl** sulfate-polyacrylamide gel **electrophoresis** (**SDS-PAGE**) and Western blot anal. Amino-terminal amino acid sequencing demonstrated a high homol. between this 97-kDa protein of swine E. **coli** O45 and the **EaeA** protein (**intimin**) of human **enteropathogenic** E. **coli** and enterohemorrhagic E. **coli**. In addn., a serol. relationship between the **EaeA** proteins of swine O45, rabbit (RDEC-1) and human (E2348/69) attaching and effacing E. coli strains was obsd. Our results indicate an assocn. between expression of the **EaeA** protein and attaching and effacing activity among O45 E. coli isolates. The data also suggest an antigenic relatedness of the **EaeA** proteins of swine, rabbit, and human attaching and effacing E. coli.

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, JICST-EPLUS, JAPIO, TOXLIT, TOXLINE, PHIC, PHIN' ENTERED AT 16:03:02 ON 26 JUL 2000)

L32 5 S L31
L33 1 S L32 NOT L8

L33 ANSWER 1 OF 1 BIOSIS COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER: 2000:88974 BIOSIS
DOCUMENT NUMBER: PREV200000088974
TITLE: Antibody response of patients infected with verocytotoxin-producing Escherichia coli to protein antigens encoded on the LEE locus.
AUTHOR(S): Jenkins, C.; Chart, H. (1); Smith, H. R.; Hartland, E. L.; Batchelor, M.; Delahay, R. M.; Dougan, G.; Frankel, G.
CORPORATE SOURCE: (1) Laboratory of Enteric Pathogens, Central Public Health Laboratory, 61 Colindale Avenue, London, NW9 5HT UK
SOURCE: Journal of Medical Microbiology, (Jan., 2000) Vol. 49, No. 1, pp. 91-101.
ISSN: 0022-2615.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Sera from patients infected with verocytotoxin-producing Escherichia coli (VTEC) O157, from patients with antibodies to E. coli O157 lipopolysaccharide (LPS) and from healthy controls were examined for antibodies to proteins involved in expressing the attaching and effacing phenotype. After **SDS-PAGE**, purified

recombinant **intimin**, EspA-filament structural protein, translocated protein EspB and three separate domains of the **translocated intimin** receptor (**Tir**) were tested for reaction with patients' sera by immunoblotting. An ELISA was also used to detect antibodies to **intimin** in sera from E. coli O157 LPS antibody-positive individuals. Seven of nine culture-positive patients and one control patient had antibodies to EspA. Five of these patients and two controls had serum antibodies to the **intimin**-binding region of **Tir**, whereas none of the sera contained antibodies binding to either of the intracellular domains of **Tir**. By immunoblotting, 10 of 14 culture-positive patients had antibodies to the conserved region of **intimin**, eight of whom were infected with E. coli O157 phage type 2. Thirty-six of 60 sera from culture-negative but E. coli O157 LPS antibody-positive patients had antibodies to **intimin** as determined by ELISA. The secreted proteins are expressed in vivo during infection and are considered as pathogenic markers. Antibodies to these proteins may form the basis of a serodiagnostic test for the detection of patients infected with VTEC which carry the locus for the enterocyte effacement pathogenicity island and provide an adjunct test to the established serological tests based on VTEC LPS.

FILE 'CAPLUS' ENTERED AT 16:05:39 ON 26 JUL 2000

L34 1 SEA ABB=ON PLU=ON (L4 OR L10) AND (SDSPAGE OR ((NA OR SODIUM)(W)DODECYL OR SDS)(5W)(PAGE OR ELECTROPHOR?))

L35 0 SEA ABB=ON PLU=ON L34 NOT (L7 OR L11 OR L31)

FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, JICST-EPLUS, JAPIO, TOXLIT, TOXLINE, PHIC, PHIN' ENTERED AT 16:06:08 ON 26 JUL 2000

L36 4 SEA ABB=ON PLU=ON L34

L37 0 SEA ABB=ON PLU=ON L36 NOT L8

FILE 'HOME' ENTERED AT 16:07:54 ON 26 JUL 2000

Devi, S.
09/189415



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      Set  Items  Description
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? ds

                                                          -key terms
Set     Items   Description
S1       4774   TIR OR TRANSLOCAT?(W)INTIMIN OR EAEA OR EAE(W)A
S2        217   S1 AND ((CITROBACTER? OR CITRO(W)BACTER? OR C)(W)RODENT? OR
                (HAFNIA OR H)(W)ALVEI OR EPEC OT ETEC OR EHEC OR ((ENTEROPAT-
                HOGEN? OR ENTEROHEMORRH? OR ENTEROHAEMORRH? OR ENTERO(W)(PATH-
                OGEN? OR HEMORRH? OR HAEMORRH?))(S)COLI))
S3          3   S2 AND (SDSPAGE OR SDS(W)PAGE)
S4         94   S2 AND INTIMIN
S5         95   S3 OR S4
S6         59   RD (unique items)
? t 6/3,ab/1-59
>>>No matching display code(s) found in file(s): 65, 113

 6/3,AB/1     (Item 1 from file: 35)
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01448642  AADAAI9540040
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ADHERENCE OF *ENTEROHEMORRHAGIC"** ESCHERICHIA *COLI"** TO HUMAN EPITHELIAL CELLS: THE ROLE OF *INTIMIN"** (BLOODY DIARRHEA, RENAL FAILURE, HEMORRHAGIC COLITIS)

Author: MCKEE, MARIAN LITTLE
Degree: PH.D.
Year: 1995
Corporate Source/Institution: UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES (0888)
Source: VOLUME 56/07-B OF DISSERTATION ABSTRACTS INTERNATIONAL. PAGE 3583. 201 PAGES

*Enterohemorrhagic"** E. *coli"** (*EHEC"**) is the leading cause of infectious bloody diarrhea in the United States as well as acute renal failure among U.S. and Canadian children. *EHEC"** colonite the large bowel and produce Shiga-like toxins (SLTs) that are considered essential for *EHEC"** virulence. *EHEC"** cause intestinal attaching and effacing (A/E) lesions in experimental animals and carry a homologue of the *enteropathogenic"** E. *coli"** (EPEC) *eaeA"** (E. *coli"** attach and efface) locus. In EPEC, the *eaeA"** gene encodes *intimin"** and is required for A/E lesion formation. To examine the importance of the *eaeA"** homologue in *EHEC"** O157:H7, we modified an existing tissue culture assay to monitor adherence to epithelial cells. With this assay, we serendipitously discovered a novel adherence phenotype, which we designated "log jam", that was shared among intestinally-derived E. *coli"**. The assay was also used to compare the in-frame *eaeA"** deletion mutation strain 86-$24eae\Delta$10 with the wild-type parent. The mutant strain, 86-$24eae\Delta$10, did not adhere to HEp-2 cells or colonize the spiral colon of infected piglets. By contrast, 86-24 formed microcolonies on HEp-2 cells, colonized the cecum and colon, and induced intestinal A/E lesions in gnotobiotic piglets. In vitro attachment and in vivo lesion formation by 86-$24eae\Delta$10 was fully restored by a clone of *EHEC"** 86-24 *eaeA"** . To further characterize *intimin"**, histidine::*intimin"** fusions were constructed and purified over a nickel affinity column. Extracts of the fusions enhanced binding of wild-type 86-24 to HEp-2 cells and conferred HEp-2 cell adherence on 86-$24eae\Delta$10 and an $*eaeA"**\sp-$ O91:H21 *EHEC"** strain. Polyclonal antisera against the His::*intimin"** fusion proteins recognized a 97 kDa outer membrane protein in *EHEC"** and EPEC. Furthermore, *intimin"**-specific antibodies blocked adherence of *EHEC"** to HEp-2 cells. These studies demonstrate that *intimin"** acts as a primary adhesin in *EHEC"** attachment to epithelial cells, is required for A/E lesion formation in the intestinal mucosa, and, as an isolated protein, confers adherence of *eaeA"**-negative strains to epithelial cells.



01378133 AAD9428400

STUDIES ON THE REGULATION OF VIRULENCE GENE EXPRESSION OF *ENTEROPATHOGENIC"** ESCHERICHIA *COLI"**

Author: GOMEZ, OSCAR GILBERTO

Degree: PH.D.
Year: 1993
Corporate Source/Institution: UNIVERSITY OF MARYLAND BALTIMORE PROFESSIONAL SCHOOLS (0373)
Source: VOLUME 55/06-B OF DISSERTATION ABSTRACTS INTERNATIONAL. PAGE 2077. 152 PAGES

*Enteropathogenic"** Escherichia *coli"** (EPEC) are a well established cause of infant diarrhea around the world, being reported primarily in developing countries and only sporadically in industrialized nations. Several genes associated with virulence have been identified. Chromosomal genes are associated with the ability of EPEC to intimately adhere to epithelial cells and to modify the cytoskeleton of enterocytes which results in effacement of microvilli. A gene known as *eaeA"** is involved in intimate adherence and encodes a 94 kDa outer membrane protein (OMP) called *intimin"**. Other genes are located in plasmids and are associated with the ability of EPEC to adhere to epithelial cells in a localized adherence pattern. Using recombinant DNA technology a region has been isolated from the EPEC plasmid which regulates the expression of *eaeA"**. This DNA region encodes a protein of 26 kDa which has been designated plasmid-encoded regulator A (PerA). DNA sequence analysis indicates that PerA has homology with the AraC family of transcriptional regulators. Two helix-turn-helix motifs have been identified at the C-terminus of PerA, suggesting that PerA is a DNA binding protein. Expression of *eaeA"** was tested under different environmental conditions by measuring the alkaline phosphatase activity of a *eaeA"**::TnphoA transposon mutant of EPEC carrying or lacking the perA gene. Temperature and exponential growth favored *eaeA"** expression in a PerA dependent fashion whereas iron proficient conditions favored *eaeA"** expression independently of PerA. Northern blot analysis shows that PerA is a positive regulator of transcription of not only *eaeA"**, but also of eaeB and bfpA. eaeB is a chromosomal gene that encodes a 39 kDa protein which is essential for intimate adherence. bfpA is a plasmid gene that encodes the 19.1 kDa protein structural subunit of the bundle forming pilus (BFP). BFP is a type IV fimbriae which is associated with localized adherence of EPEC to epithelial cells. In addition to *eaeA"**, eaeB, and bfpA genes PerA may also regulate other genes as indicated by OMP analysis. Two OMPs of 50 kDa and 33 kDa are overexpressed in $perA\sp+$ strains whereas the expression of a 20 kDa OMP is decreased. Differential expression of OMPs indicates that PerA may act as a positive, as well as a negative regulator of EPEC genes. DNA sequence analysis has identified two direct repeats in the promoter regions of both *eaeA"** and eaeB genes which may serve as potential binding sites for PerA. The ability of PerA to affect the expression of a variety of virulence associated genes suggests that PerA is a global regulator of virulence gene expression which may be essential in EPEC pathogenesis.

6/3,AB/3 (Item 1 from file: 65)
DIALOG(R)File 65:Inside Conferences


02468319 INSIDE CONFERENCE ITEM ID: CN025774597
BipA affects Ca SUP 2 SUP + fluxes and phosphorylation of the *translocated"** *intimin"** receptor (*Tir"**/Hp90) in host epithelial cells infected with *enteropathogenic"** Escherichia *coli"**
Farris, M.; Grant, A.; Jane, S.; Chad, J. E.
CONFERENCE: Biochemical Society-Meeting; 665th
BIOCHEMICAL SOCIETY TRANSACTIONS, 1998; VOL 26; NUMBER 3 P: S225
London, Portland Press, 1998
ISSN: 0300-5127
LANGUAGE: English DOCUMENT TYPE: Conference Papers and extended abstracts
CONFERENCE SPONSOR: Biochemical Society
CONFERENCE LOCATION: Southampton
CONFERENCE DATE: Mar 1998 (199803) (199803)

6/3,AB/4 (Item 1 from file: 144)
DIALOG(R)File 144:Pascal


14593338 PASCAL No.: 00-0261142
Role of *tir"** and *intimin"** in the virulence of rabbit *enteropathogenic"** Escherichia *coli"** serotype O103:H2
MARCHES O; NOUGAYREDE J P; BOULLIER S; MAINIL J; CHARLIER G; RAYMOND I; POHL P; BOURY M; DE RYCKE J; MILON A; OSWALD E
Unite Mixte de Microbiologie Moleculaire, Institut National de la Recherche Agronomique-Ecole Nationale Veterinaire de Toulouse, 31076 Toulouse, France; Chaire de Bacteriologie et de Pathologie des Maladies Bacteriennes, Faculte de Medecine Veterinaire, Universite de Liege, 4000 Liege, Belgium; Centre d'Etudes et de Recherches Veterinaires et Agrochimiques, 1180 Brussels, Belgium; Laboratoire d'Anatomie Pathologique, Ecole Nationale Veterinaire de Toulouse, 31076 Toulouse, France
Journal: Infection and immunity, 2000, 68 (4) 2171-2182
Language: English
Attaching and effacing (A/E) rabbit *enteropathogenic"** Escherichia *coli"** (REPEC) strains belonging to serogroup 0103 are an important cause of diarrhea in weaned rabbits. Like human EPEC strains, they possess the locus of enterocyte effacement clustering the genes involved in the formation of the A/E lesions. In addition, pathogenic REPEC 0103 strains produce an Esp-dependent but Eae (*intimin"**)-independent alteration of the host cell cytoskeleton characterized by the formation of focal adhesion complexes and the reorganization of the actin cytoskeleton into bundles of stress fibers. To investigate the role of *intimin"** and its translocated

coreceptor (*Tir"** ) in the pathogenicity of REPEC, we have used a newly constructed isogenic *tir"** null mutant together with a previously described eae null mutant. When human HeLa epithelial cells were infected, the *tir"** mutant was still able to induce the formation of stress fibers as previously reported for the eae null mutant. When the rabbit epithelial cell line RK13 was used, REPEC 0103 produced a classical fluorescent actin staining (FAS) effect, whereas both the eae and *tir"** mutants were FAS negative. In a rabbit ligated ileal loop model, neither mutant was able to induce A/E lesions. In contrast to the parental strain, which intimately adhered to the enterocytes and destroyed the brush border microvilli, bacteria of both mutants were clustered in the mucus without reaching and damaging the microvilli. The role of *intimin"** and *Tir"** was then analyzed in vivo by oral inoculation of weaned rabbits. Although both mutants were still present in the intestinal flora of the rabbits 3 weeks after oral inoculation, neither mutant strain induced any clinical signs or significant weight loss in the inoculated rabbits whereas the parental strain caused the death of 90% of the inoculated rabbits. Nevertheless, an inflammatory infiltrate was present in the lamina propria of the rabbits infected with both mutants, with an inflammatory response greater for the eae null mutant. In conclusion, we have confirmed the role of *intimin"** in virulence, and we have shown, for the first time, that *Tir"** is also a key factor in vivo for pathogenicity.



6/3,AB/5 (Item 2 from file: 144)

DIALOG(R)File 144:Pae their *intimin"**-encoding (eae) gene with probes derived from the variable parts of the eae alpha gene of the human EPEC strain E2348/69, the eae gamma gene of the human 0157:H7 *EHEC"** strain ATCC43888, and the eae beta gene of the bovine 026:H- *EHEC"** strain 193, whose eae gene was first cloned and sequenced during this work. The EPEC and *EHEC"** had been isolated from diarrhoeic calves (143 EPEC and 48 *EHEC"**) and from healthy animals at the slaughterhouse (10 EPEC and 34 *EHEC"**). The 191 bovine EPEC and *EHEC"** isolated from diseased calves were positive with the Eae beta probe (55 and 27% respectively) and with the Eaey probe (9 and 73% respectively), whereas 52 EPEC (36%) were negative with the *Eaea"**, Eae beta , and Eaey probes. The results were different for the 44 bovine EPEC and *EHEC"** isolated from healthy cattle at slaughterhouses: most tested positive with the Eaey probe (80 and 82% respectively) and the remaining (20 and 18% respectively) with the Eae beta probe. Nine 026 human *EHEC"** tested positive with the Eae beta probe and seven 0111 with the Eaey probe. The bovine and human EPEC and *EHEC"** belonging to these two serogroups gave identical results: the 18 bovine and human 026 isolates tested positive with the Eae beta probe, whereas the 13 0111 isolates were positive with the Eaey probe. In contrast, the isolates belonging to other serogroups (05, 015, 018, 020, and 0118) gave more variable results. The eae beta and eae gamma , but not the eae alpha , variants were thus distributed amongst bovine EPEC and *EHEC"**. The eae

beta variant seemed to be more frequently associated with the presence of clinical signs in calves, but one third of EPEC from diarrhoeic calves carried an eae gene variant other than the alpha , beta , or y variants. In addition, the use of these gene probes did not enable differentiation between bovine and human *EHEC"** belonging to the same 0 serogroup.



6/3,AB/8 (Item 5 from file: 144)
DIALOG(R)File 144:Pascal


14116878 PASCAL No.: 99-0312625

*Enterohemorrhagic"** Escherichia *coli"** O157:H7 produces *Tir"**, which is translocated to the host cell membrane but is not tyrosine phosphorylated

DEVINNEY R; STEIN M; REINSCHEID D; ABE A; RUSCHKOWSKI S; FINLAY B B
Biotechnology Laboratory, University of British Columbia, Vancouver, British Columbia V6T 1Z3, Canada

Journal: Infection and immunity, 1999, 67 (5) 2389-2398
Language: English

Intimate attachment to the host cell leading to the formation of attaching and effacing (A/E) lesions is an essential feature of *enterohemorrhagic"** Escherichia *coli"** (*EHEC"**) O157:H7 pathogenesis. In a related pathogen, *enteropathogenic"** E. *coli"** (EPEC), this activity is dependent upon translocation of the *intimin"** receptor, *Tir"** , which becomes tyrosine phosphorylated within the host cell membrane. In contrast, the accumulation of tyrosine-phosphorylated proteins beneath adherent *EHEC"** bacteria does not occur, leading to questions about whether *EHEC"** uses a *Tir"**-based mechanism for adherence and A/E lesion formation. In this report, we demonstrate that *EHEC"** produces a functional *Tir"** that is inserted into host cell membranes, where it serves as an *intimin"** receptor. However, unlike in EPEC, in *EHEC"** *Tir"** is not tyrosine phosphorylated yet plays a key role in both bacterial adherence to epithelial cells and pedestal formation. *EHEC"**, but not EPEC, was unable to synthesize *Tir"** in Luria-Bertani medium but was able to secrete *Tir"** into M9 medium, suggesting that *Tir"** synthesis and secretion may be regulated differently in these two pathogens. *EHEC"** *Tir"** and EPEC *Tir"** both bind *intimin"** and focus cytoskeletal rearrangements, indicating that tyrosine phosphorylation is not needed for pedestal formation. *EHEC"** and EPEC intimins are functionally interchangeable, but *EHEC"** *Tir"** shows a much greater affinity for *EHEC"** *intimin"** than for EPEC *intimin"**. These findings highlight some of the differences and similarities between *EHEC"** and EPEC virulence mechanisms, which can be exploited to further define the molecular basis of pedestal formation.

6/3,AB/9 (Item 6 from file: 144)
DIALOG(R)File 144:Pascal


14064589 PASCAL No.: 99-0256206
Relationship between O-serogroup and presence of pathogenic factor genes in Escherichia coli
SUNABE T; HONMA Y
Department of Bacteriology, Faculty of Medicine, University of the Ryukyus, Okinawa 903-0215, Japan
Journal: Microbiology and immunology, 1998, 42 (12) 845-849
Language: English
A total of 383 isolates of serogroup-based *enteropathogenic"** and enteroinvasive Escherichia *coli"** (310 strains of EPEC and 73 strains of EIEC) were examined for the presence of corresponding pathogenic genes. The serogroup-based EPEC consisted of 232 strains isolated from diarrhea patients and of 78 strains from healthy carriers. The gene encoding *intimin"**, *eaeA"** , was detected in 42 of the 232 EPEC strains from patients (18.1%) and 9 of the 78 strains from carriers (11.5%). The difference was not significant. The bfp gene on the EAF plasmid was detected in 7 of the 42 *eaeA"**-positive EPEC strains from patients but was not detected in the 9 strains from carriers. In serogroup-based EIEC, a chromosomal ipaH gene encoding one of the invasive plasmid antigens was detected in 4 of the 60 strains from patients (6%) but not in the 13 strains from carriers. The 4 ipaH-positive strains possessed the invasive plasmid. These results suggested that the serogroup-based diagnosis of EPEC and EIEC is not sufficient for identifying strains carrying the *eaeA"** or ipaH gene.



6/3,AB/10 (Item 7 from file: 144)
DIALOG(R)File 144:Pascal


14033420 PASCAL No.: 99-0222578
Detection of shiga-like toxin (stx SUB 1 and stx SUB 2 ), *intimin"** ( *eaeA"**), and *enterohemorrhagic"** escherichia *coli"** (*EHEC"**) hemolysin (*EHEC"** hlyA) genes in animal feces by multiplex PCR
FAGAN P K; HORNITZKY M A; BETTELHEIM K A; DJORDJEVIC S P
Elizabeth Macarthur Agricultural Institute, New South Wales Agriculture, Camden, New South Wales 2570, Australia; Victorian Infectious Diseases Reference Laboratory, Fairfield Hospital, Fairfield, Victoria 3078, Australia
Journal: Applied and environmental microbiology, 1999, 65 (2) 868-872
Language: English

A multiplex PCR was developed for the rapid detection of genes encoding Shiga toxins I and 2 (stx SUB 1 and stx SUB 2 ), *intimin"** (*eaeA"**), and enterohemolysin A (hlyA) in 444 fecal samples derived from healthy and clinically affected cattle, sheep, pigs, and goats. The method involved non-solvent-based extraction of nucleic acid from an aliquot of an overnight culture of feces in EC (modified) broth. The detection limit of the assay for both fecal samples and pure cultures was between 18 and 37 genome equivalents, stx SUB 1 and hlyA were the most commonly encountered virulence factors.



6/3,AB/11 (Item 8 from file: 144)
DIALOG(R)File 144:Pascal


13999988 PASCAL No.: 99-0184953
*Enteropathogenic"** Escherichia *coli"** inhibits phagocytosis
GOOSNEY D L; CELLI J; KENNY B; FINLAY B B
Biotechnology Laboratory and Departments of Microbiology & Immunology and of Biochemistry & Molecular Biology, University of British Columbia, Vancouver, British Columbia V6T 1Z3, Canada
Journal: Infection and immunity, 1999, 67 (2) 490-495
Language: English

*Enteropathogenic"** Escherichia *coli"** (EPEC) interacts with intestinal epithelial cells, activating host signaling pathways leading to cytoskeletal rearrangements and ultimately diarrhea. In this study, we demonstrate that EPEC interacts with the macrophage-like cell line J774A.1 to inhibit phagocytosis by these cells. Antiphagocytic activity was also observed in cultured RAW macrophage-like cells upon EPEC infection. The EPEC antiphagocytic phenotype was dependent on the type III secretion pathway of EPEC and its secreted proteins, including EspA, EspB, and EspD. *Intimin"** and *Tir"** mutants displayed intermediate antiphagocytic activity, suggesting that intimate attachment mediated by *intimin"**-*Tir"** binding may also play a role in antiphagocytosis. Tyrosine dephosphorylation of several host proteins was observed following infection with secretion-competent EPEC but not with secretion-deficient mutants. Dephosphorylation was detectable 120 min after infection with EPEC, directly correlating with the onset of the antiphagocytic phenotype. Inhibition of protein tyrosine phosphatases by pervanadate treatment increased the number of intracellular wild-type EPEC organisms to levels seen with secretion-deficient mutants, suggesting that dephosphorylation events are linked to the antiphagocytic phenotype. No tyrosine phosphatase activity was detected with the EPEC-secreted proteins, suggesting that EPEC induces antiphagocytosis via a different mechanism than Yersinia species. Taken together, the present findings demonstrate a novel function for EPEC-secreted proteins in triggering macrophage protein tyrosine dephosphorylation and inhibition of phagocytosis.

6/3,AB/12 (Item 9 from file: 144)
DIALOG(R)File 144:Pascal


13909173 PASCAL No.: 99-0090002
Molecular and ultrastructural characterisation of EspA from different *enteropathogenic"** Escherichia *coli"** serotypes
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Journal: FEMS microbiology letters, 1998, 169 (1) 73-80
Language: English
*Enteropathogenic"** Escherichia *coli"** (EPEC) encode a type III secretion system located on a pathogenicity island known as the locus for enterocyte effacement. Four proteins are known to be exported by this type III secretion system EspA, EspB and EspD required for subversion of host cell signal transduction pathways and a *translocated"** *intimin"** receptor protein (*Tir"**) required for *intimin"**-mediated intimate attachment and attaching and effacing lesion formation. The espA gene is located within the locus for enterocyte effacement and the EspA polypeptide from the prototype EPEC strain E2348/69 (O127:H6) has recently been shown to be a component of a filamentous structure involved in bacteria-host cell interaction and locus for enterocyte effacement-encoded protein translocation involved in attaching and effacing lesion formation. In this study we have extended our investigation of EspA to strains belonging to other classical EPEC serotypes. DNA sequencing demonstrated that the espA gene from the different EPEC strains share at least 65% DNA identity. In addition, we detected morphologically and antigenically similar EspA filaments in all but one of the bacterial strains examined including recombinant, non-pathogenic E. *coli"** expressing espA from a cloned locus for enterocyte effacement region (HB101(pCVD462)).



6/3,AB/13 (Item 10 from file: 144)
DIALOG(R)File 144:Pascal

13868156 PASCAL No.: 99-0046090

*Translocated"** *intimin"** receptors (*Tir"**) of shiga-toxigenic Escherichia coli isolates belonging to serogroups O26, O111, and O157 react with sera from patients with hemolytic-uremic syndrome and exhibit marked sequence heterogeneity

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Journal: Infection and immunity, 1998, 66 (11) 5580-5586

Language: English

The capacity to form attaching and effacing (A/E) lesions on the surfaces of enterocytes is an important virulence trait of several enteric pathogens, including *enteropathogenic"** Escherichia *coli"** (EPEC) and Shiga-toxigenic E. *coli"** (STEC). Formation of such lesions depends upon an interaction between a bacterial outer membrane protein (*intimin"**) and a bacterially encoded receptor protein (*Tir"**) which is exported from the bacterium and translocated into the host cell membrane. *Intimin"**, *Tir"**, and several other proteins necessary for generation of A/E lesions are encoded on a chromosomal pathogenicity island termed the locus for enterocyte effacement (LEE). Reports of sequence heterogeneity and antigenic variation in the region of *intimin"** believed to be responsible for receptor binding raise the possibility that the receptor itself is also heterogeneous. We have examined this by cloning and sequencing *tir"** genes from three different STEC strains belonging to serogroups 026, 0111, and O157. The deduced amino acid sequences for the *Tir"** homologues from these strains varied markedly, exhibiting only 65.4, 80.2, and 56.7% identity, respectively, to that recently reported for EPEC *Tir"**. STEC *Tir"** is also highly immunogenic in humans. Western blots of E. *coli"** DH5 alpha expressing the various STEC *tir"** genes cloned in pBluescript (but not E. *coli"** DH5 alpha (pBluescript)) reacted strongly with convalescent sera from patients with hemolytic-uremic syndrome (HUS) caused by known LEE-positive STEC. Moreover, no reaction was seen when the various clone lysates were probed with serum from a patient with HUS caused by a LEE-negative STEC or with serum from a healthy individual. Covariation of exposed epitopes on both *intimin"** and *Tir"** may be a means whereby STEC avoid host immune responses without compromising adhesin-receptor interaction.



6/3,AB/14 (Item 11 from file: 144)

DIALOG(R)File 144:Pascal



13556848 PASCAL No.: 98-0258526

Molecular analysis of Shiga toxigenic Escherichia coli O111:H SUP -

proteins which react with sera from patients with hemolytic-uremic syndrome

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Journal: Infection and immunity, 1998, 66 (4) 1467-1472

Language: English

Western blot analysis was used to assess the reactivity of convalescent-phase sera from patients who were associated with an outbreak of hemolytic-uremic syndrome (HUS) caused by fermented sausage contaminated with Shiga toxin-producing Escherichia *coli"** (STEC). The predominant STEC isolated from HUS patients belonged to serotype O111:H SUP - , and reactivity to O111:H SUP - whole-cell lysates, treated or untreated with proteinase K, was examined. As expected, all five serum samples demonstrated a marked anti-lipopolysaccharide response, but several protein bands were also immunoreactive, particularly one with an apparent size of 94 kDa. One convalescent-phase serum sample was subsequently used to screen an O111:H SUP - cosmid bank and 2 of 900 cosmid clones were found to be positive, both of which contained a similar DNA insert. Western blot analysis of one of these clones identified three major immunoreactive protein bands of approximately 94, 70, and 50 kDa. An immune response to the three proteins was detectable with all five convalescent-phase serum samples but not with healthy human serum. Immunoreactive 94- and 50-kDa species were produced by a deletion derivative of the cosmid containing a 7-kb STEC DNA insert. Sequence analysis of this region indicated that it is part of the locus for enterocyte effacement, including the *eaeA"** gene which encodes *intimin"** . The deduced amino acid sequence of the O111:H SUP - *intimin"** was 88.6% identical to *intimin"** from O157:H7 STEC, and the most divergent region was the 200 residues at the carboxyl terminus, which were only 75% identical. Such variation may be antigenically significant as serum from a HUS patient infected only with the O111:H SUP - STEC reacted with *intimin"** from an *enteropathogenic"** E. *coli"** O111 strain, as well as several other *eaeA"**-positive STEC isolates, but not with an *eaeA"** -positive STEC belonging to serotype O157:H SUP - . Sera from two of the other HUS patients also failed to react with *intimin"** from this latter strain. However, *intimin"** from O157:H SUP - STEC did react with serum from a patient infected with both O111:H SUP - and O157:H SUP - STEC.



6/3,AB/15 (Item 12 from file: 144)

DIALOG(R)File 144:Pascal



13302601 PASCAL No.: 98-0026409

Flagellar flhA, flhB and flhE genes, organized in an operon, cluster

upstream from the inv locus in Yersinia enterocolitica
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Journal: Microbiology : (Reading), 1997, 143 (p.11) 3461-3471
Language: English
The inv gene of Yersinia enterocolitica codes for invasin, a member of the invasin/*intimin"** -like protein family, which mediates the internalization of the bacterium into cultured epithelial cells. The putative inclusion of inv into a pathogenicity island was tested by investigating its flanking sequences. Indeed, the *enteropathogenic"** Escherichia *coli"** (EPEC) *intimin"**, a member of the same family of proteins, is encoded by *eaeA"**, a gene which belongs to a pathogenicity island. An ORF located upstream from inv was of particular interest since it appeared homologous both to the flagellar flhA gene and to sepA, an EPEC gene lying inside the same pathogenicity island as *eaeA"**. A mutant in this ORF was non-motile and non-flagellated while its invasion phenotype remained unaffected. These data indicated that the ORF corresponded to the flhA gene of Y. enterocolitica. Subsequently, the flhB and flhE genes, located respectively upstream and downstream from flhA, were identified. The three flh genes appear to be transcribed from a single operon called flhB, according to the nomenclature used for Salmonella typhimurium. Intergenic sequence between flhE and inv includes a grey hole, with no recognizable function. Downstream from inv, we have detected the flagellar flgM operon as already reported. Finally, the incongruous localization of inv amidst the flagellar cluster is discussed; while transposition could explain this phenomenon, no trace of such an event was detected.



6/3,AB/16 (Item 13 from file: 144)
DIALOG(R)File 144:Pascal


13033258 PASCAL No.: 97-0319142
Genetic and phenotypic analysis of Escherichia *coli"** with *enteropathogenic"** characteristics isolated from Seattle children
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Journal: The Journal of infectious diseases, 1997, 175 (6) 1382-1389
Language: English

Coliform colonies from children whose stools were submitted for microbiologic analysis were studied prospectively to determine the frequency of shedding of *enteropathogenic"** Escherichia *coli"** (EPEC). In total, 2225 isolates from 445 patients were probed with *eaeA"** (encoding *intimin"** ) and the EAF (EPEC adherence factor) probe, and adherence and actin-aggregating phenotypes were determined. Twenty-five patients (5.6%) shed non-O157:H7 *eaeA"** SUP + EAF SUP - E. *coli"**. Of these 25 patients, isolates from 5 produced Shiga toxins and from 3 possessed bfpA (encoding the bundle-forming pilus) sequences. Non-O157:H7 *eaeA"** SUP + E. *coli"** from 21 (84%) of 25 patients adhered locally to and aggregated actin in HeLa cells. Four patients shed nonadherent EAF SUP + *eaeA"** SUP - E. *coli"**. Non-O157:H7 *eaeA"** SUP + and EAF SUP + isolates belonged to diverse electrophoretic types and classical and nonclassical *enteropathogenic"** serotypes. EPEC are relatively common in stools submitted for analysis in this North American pediatric hospital. Their etiologic role in childhood diarrhea warrants elucidation.



6/3,AB/17 (Item 14 from file: 144)
DIALOG(R)File 144:Pascal


12991030 PASCAL No.: 97-0270738
Down regulation of *intimin"** expression during attaching and effacing *enteropathogenic"** Escherichia *coli"** adhesion
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Journal: Infection and immunity, 1997, 65 (5) 1644-1652
Language: English

*Enteropathogenic"** Escherichia *coli"** (EPEC) produces attaching and effacing (A/E) lesions in the intestinal mucosa. The intimate bacterial adhesion associated with A/E lesion formation is promoted by *intimin"**, a 94-kDa EPEC surface protein. Anti-*intimin"** antisera raised in rabbits by using the purified 280-amino-acid cell binding domain of *intimin"** as the immunogen were employed in immunofluorescence and immunoelectron microscopical studies to investigate the expression of *intimin"** by classical EPEC strain E2348/69 (O127:H6) and defined E2348/69 derivatives during culture growth and A/E bacterium adhesion to cultured HEp-2 cells. In stationary-phase broth cultures, only a small fraction of E2348/69 bacteria expressed *intimin"** , and of those that did, immunolabelling revealed a uniform distribution of *intimin"** over the bacterial surface;

increased numbers of bacteria expressing *intimin"** were detected when E2348/69 was grown in tissue culture medium, an effect not seen with strain JPN15, a virulence plasmid-cured derivative of E2348/69. Strain CVD206, an *eaeA"** mutant of E2348/69, did not stain with the anti-*intimin"** antisera, but strain CVD206(pCVD438), containing a functional *eaeA"** gene, stained uniformly. After a 3-h incubation of HEp-2 cells with strain E2348/69, double immunofluorescence labelling of *intimin"** and cellular actin revealed strong *intimin"** expression by all A/E bacteria, but after 6 h of incubation, *intimin"** expression by most E2348/69 bacteria was greatly reduced or not detected. This effect on *intimin"** expression was not observed with strain JPNIS but was restored for strain JPN15(pCVD450) harboring the virulence plasmid-encoded per genes. These results indicate that surface expression of *intimin"** is regulated by environmental factors during bacterial growth and following A/E lesion formation and that virulence plasmid-encoded genes participate in these regulation processes.



6/3,AB/18 (Item 15 from file: 144)
DIALOG(R)File 144:Pascal


12845165 PASCAL No.: 97-0065216
Identification of a family of intimins common to Escherichia coli causing attaching-effacing lesions in rabbits, humans, and swine
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Journal: Infection and immunity, 1997, 65 (1) 320-326
Language: English
*Intimin"**, an outer membrane protein encoded by *eaeA"** that mediates close attachment of *enteropathogenic"** bacteria to apical surfaces of epithelial cells, is required for formation of the attaching-effacing lesions and for full pathogenesis of the bacteria. Analysis of the *eaeA"** sequence indicates that there is a high degree of homology at the N termini but less at the C termini of intimins. Antisera specific for the C-terminal third of RDEC-1 *intimin"**, used to screen outer membrane proteins from 50 rabbit *enteropathogenic"** Escherichia *coli"** (EPEC), human EPEC, and human *enterohemorrhagic"** E. *coli"** (*EHEC"** ) strains, identified cross-reactive intimins from 24 isolates. Sequence analysis of the *eaeA"** genes from human EPEC 0111 and *EHEC"** 026 isolates indicates that their intimins have C termini nearly identical to that of RDEC-1 *intimin"**. Our results suggest that there are at least three families of related intimins and that the presence of *intimin"** similar to that of RDEC-1 is not restricted by serogroup or host specificity.

6/3,AB/19 (Item 16 from file: 144)
DIALOG(R)File 144:Pascal


12824434 PASCAL No.: 97-0041176

*Intimin"** from *enteropathogenic"** Escherichia *coli"** restores murine virulence to a *Citrobacter"** *rodentium"** *eaeA"** mutant : Induction of an immunoglobulin A response to *intimin"** and EspB

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Journal: Infection and immunity, 1996, 64 (12) 5315-5325

Language: English

The formation of attaching and effacing (A/E) lesions is central to the pathogenesis of *enteropathogenic"** Escherichia *coli"** (EPEC)-mediated disease in humans and *Citrobacter"** *rodentium"** (formerly C. freundii biotype 4280)-mediated transmissible colonic hyperplasia in mice. Closely related outer membrane proteins, known as intimins, are required for formation of the A/E lesion by both EPEC (Int SUB E SUB P SUB E SUB C ) and *C"**. *rodentium"** (Int SUB C SUB R ). A secreted protein, EspB (formally EaeB), is also necessary for A/E-lesion formation. Here we report that expression ofa cloned Int SUB E SUB P SUB E SUB C , encoded by plasmid pCVD438, restores murine virulence to an *intimin"**-deficient mutant of *C"**. *rodentium"** DBS255. Replacement of Cys937 with Ala abolished the ability of the cloned EPEC *intimin"** to complement the deletion mutation in DBS255. Ultrastructural examination of tissues from wild-type *C"**. *rodentium"** and DBS255(pCVD438)-infected mice revealed multiple A/E lesion on infected cells and loss of contact between enterocytes and basement membrane. Histological investigation showed that although both wild-type *C"**. *rodentium"** and DBS255(pCVD438) colonized the descending colon and induced colonic hyperplasia in orally infected 21-day-old mice, the latter strain adhered to epithelial cells located deeper within crypts. Nonetheless, infection with the wild-type strain was consistently more virulent, as indicated by a higher mortality rate. All the surviving mice, challenged with either wild-type *C"**. *rodentium"** or DBS255(pCVD438), developed a mucosal immunoglobulin A response to *intimin"** and EspB. These results show that *C"**. *rodentium"** infection provides a relevant, simple, and economic model to investigate the role of EPEC proteins in the formation of A/E lesions in vivo and in intestinal disease.

6/3,AB/20 (Item 17 from file: 144)
DIALOG(R)File 144:Pascal


12780594 PASCAL No.: 96-0499779
Characterization of the *eaeA"** gene from rabbit *enteropathogenic"** Escherichia *coli"** strain RDEC-1 and comparison to other *eaeA"** genes from bacteria that cause attaching-effacing lesions
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Journal: FEMS microbiology letters, 1996, 144 (2-3) 249-258
Language: English
A number of enteric pathogens, including *enteropathogenic"** (EPEC) and *enterohemorrhagic"** (*EHEC"**) Escherichia *coli"**, *Hafnia"** *alvei"** , a strain of Citrobacter freundii, and rabbit EPEC strain RDEC-1 cause attaching-effacing (AE) lesions in the gut mucosa. These bacteria have a pathogenicity cassette (locus of enterocyte effacement or LEE) containing the *eaeA"** gene. This gene encodes *intimin"**, an outer membrane protein required for production of AE lesions. RDEC-1, a non-invasive *enteropathogen"** in young rabbits, produces AE lesions morphologically indistinguishable from lesions caused by human AE bacterial strains. The RDEC-1 example of E. *coli"** diarrhea in rabbits is an important model for studying the pathogenesis of AE bacteria in a natural infection and for analyzing specific roles of the components of LEE. In order to better understand the role of *intimin"** in the development of AE lesions, a portion of DNA within RDEC-1 LEE, containing the *eaeA"** gene and an upstream open reading frame (ORF), was sequenced. The RDEC-1 *eaeA"** gene shared 87%, 92%, and 93% DNA sequence identity and > 80% amino acid sequence identity with the *eaeA"** genes of C. freundii biotype 4280, *EHEC"** O157:H7, and EPEC O127:H6, respectively. The carboxy-terminal 280 amino acid residues of *intimin"** has 80%, 56%, and 54% identity with C. freundii, *EHEC"** O157:H7, and EPEC O127:H6 intimins, respectively. The predicted protein encoded by the upstream ORF (156 amino acids) shares 95%, 97%, and 99% amino acid identity with predicted proteins from C. freundii, *EHEC"** O157:H7, and EPEC O127:H6, respectively. The high degree of sequence homology of the ORF and the *eaeA"** gene of RDEC-1 with those of other AE bacteria suggests an evolutionary relationship of LEE and supports and facilitates the use of the RDEC-1 model for studying the role of LEE in pathogenesis.



6/3,AB/21 (Item 18 from file: 144)
DIALOG(R)File 144:Pascal

12640376 PASCAL No.: 96-0334089
Clonal structure and virulence factors in strains of Escherichia coli of the classic serogroup O55
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Journal: Infection and immunity, 1996, 64 (7) 2680-2686
Language: English
Virulence properties and genetic variation as determined by multilocus enzyme electrophoresis were studied in 70 strains of Escherichia *coli"** O55, a common serogroup of *enteropathogenic"** E. *coli"** (EPEC), a major cause of infantile diarrhea in developing countries. Nearly 40% of the strains were originally isolated in Brazil and represented serotypes O55 :H6, O55 :H7, and O55 :H51 and nonmotile (O55 :H-) strains. The analysis of electrophoretic variants of 20 enzymes defined seven distinct electrophoretic types (ETs). ET1 was represented by 41% of the strains, including strains which usually hybridized with DNA probes for the *intimin"** gene (*eaeA"**), the EPEC adherence plasmid (EAF), and the gene for the pilin subunit of the bundle-forming pilus (bfpA). The ET 1 strains were also typically serotype O55 :H6, displayed localized adherence (LA) in tissue culture assays, and were positive in the fluorescent-actin staining test for intimate cell adherence. These same characteristics were observed in the closely related ETs 2 to 4, which clustered in the same branch as ET 1. No known virulence marker could be identified in ET 6. ET 5 included 23 strains, all of which carried the *eaeA"** gene but otherwise displayed a striking array of distinct virulence traits. This ET was represented by O55 :H7 strains with phenotypes as diverse as the simultaneous expression of LA and diffuse adherence and the ability to form a newly described adherence pattern, called LA-like adherence. The results suggest that ET 5 marks a special pathogenic clone with a propensity to acquire virulence factors which may facilitate the emergence of new pathogenic strains.

6/3,AB/22 (Item 19 from file: 144)
DIALOG(R)File 144:Pascal


12597587 PASCAL No.: 96-0284752
Truncated *enterohemorrhagic"** Escherichia *coli"** (*EHEC"**) O157:H7 *intimin"** (*EaeA"**) fusion proteins promote adherence of *EHEC"** strains to HEp-2 cells
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Journal: Infection and immunity, 1996, 64 (6) 2225-2233
Language: English
*Intimin"**, the product of the *eaeA"** gene in *enterohemorrhagic"**

Escherichia *coli"** O157 :H7 (*EHEC"** ), is required for intimate adherence of these organisms to tissue culture cells and formation of the attaching and effacing lesion in the gnotobiotic pig. Because of the importance of *intimin"** in the pathogenesis of *EHEC"** O157 :H7 infection in this animal model, we began a structure-function analysis of *EaeA"**. For this purpose, we constructed amino-terminal fusions of the *intimin"** protein with six histidine residues to form two independent fusions. The longer fusion, RIHisEae, contained 900 of the 935 predicted amino acids and included all but the extreme amino terminus. The second fusion, RVHdHisEae, consisted of the carboxyl two-thirds of the protein. Purified extracts of either construct enhanced binding of wild-type 86-24 to HEp-2 cells and conferred HEp-2 cell adherence on 86-24eae DELTA 10, an *eaeA"** deletion mutant, and B2F1, an *EHEC"** O91 :H21 *eaeA"** mutant strain. When 86-24eae DELTA 10 was transformed with either of the plasmids encoding the *intimin"** fusion proteins, the transformant behaved like the wild-type parent strain and displayed localized adherence to HEp-2 cells, with positive fluorescent-actin staining. In addition, polyclonal antisera raised against RIHisEae reacted with both fusion constructs and recognized an outer membrane protein of the same mass as *intimin"** (97 kDa) in *EHEC"** and *enteropathogenic"** E. *coli"** but not E. *coli"** K-12. The *intimin"**-specific antisera also blocked adherence of *EHEC"** to HEp-2 cells. Thus, *intimin"** (i) is a 97-kDa outer membrane protein in *EHEC"** that serves as a requisite adhesin for attachment of the bacteria to epithelial cells, even when the protein is truncated by one-third at its amino terminus and (ii) can be added exogenously to specifically facilitate HEp-2 cell adherence of *EHEC"** but not E. *coli"** K-12.

6/3,AB/23 (Item 20 from file: 144)
DIALOG(R)File 144:Pascal


12518297 PASCAL No.: 96-0192036
Genotypic and phenotypic characterization of Escherichia coli isolates from dogs manifesting attaching and effacing lesions
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Journal: Journal of clinical microbiology, 1996, 34 (1) 144-148
Language: English
Thirteen Escherichia *coli"** isolates from dogs manifesting attaching and effacing lesions were characterized genetically with respect to the presence of the following virulence determinants associated with human *enteropathogenic"** E. *coli"** (EPEC) : *eaeA"**, encoding the outer membrane proteins *intimin"** ; eaeB, which is necessary for inducing signal transduction ; bfpA, encoding the bundle-forming pilus ; and the EAF (stands for EPEC adherence factor) plasmid. These isolates were also analyzed phenotypically with respect to adherence to mammalian cells in

vivo and in vitro. Nine of these 13 isolates were found to be *eaeA"** positive by PCR ; four of these nine were eaeB positive. The 5' end, but not the 3' end, of the *eaeA"** gene was amplified by PCR when primers derived from the *eaeA"** gene of EPEC were used. Six and eight of these 13 isolates were found to be bfpA positive and EAF positive, respectively. The bfpA gene and EAF locus were found on high-molecular-weight plasmids, whereas the *eaeA"** and eaeB genes were chromosomally located when present. Only one canine E. *coli"** isolate, 4221, which was positive for *eaeA"**, eaeB, bfpA, and EAF, adhered to HEp-2 cells in a localized manner and was positive in the fluorescence actin staining test. The nine *eaeA"** -positive isolates adhered to the mucosal surface of piglet ileal explants and induced some microvillus effacement. However, when tested in experimentally inoculated gnotobiotic piglets, isolate 4221 did not induce attaching and effacing lesions at any level of the intestinal tract. Our results indicate that canine E. *coli"** isolates associated with attaching and effacing lesions share some properties with human EPEC but form a heterogeneous group.

6/3,AB/24 (Item 21 from file: 144)
DIALOG(R)File 144:Pascal


12273651 PASCAL No.: 95-0503777
*Enterohemorrhagic"** Escherichia *coli"** O157:H7 requires *intimin"** to colonize the gnotobiotic pig intestine and to adhere to HEp-2 cells
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Journal: Infection and immunity, 1995, 63 (9) 3739-3744
Language: English
In a previous study, *enterohemorrhagic"** Escherichia *coli"** (*EHEC"** ) O157 :H7 with a deletion and insertion in the *eaeA"** gene encoding *intimin"** was used to establish that *intimin"** is required for the organism to attach to and efface microvilli in the piglet intestine (M. S. Donnenberg, S. Tzipori, M. L. McKee, A. D. O'Brien, J. Alroy, and J. B. Kaper, J. Clin. Invest. 92 :1418-1424, 1993). However, in the same investigation, a role for *intimin"** in *EHEC"** adherence to HEp-2 cells could not be definitively demonstrated. To analyze the basis for this discrepancy, we constructed an in-frame deletion of *eaeA"** and compared the adherence capacity of this mutant with that of the wild-type strain in vitro and in vivo. We observed a direct correlation between the requisite for *intimin"** in *EHEC"** O157 :H7 colonization of the gnotobiotic piglet intestine and adherence of the bacterium to HEp-2 cells. The in vitro-in vivo correlation lends credence to the use of the HEp-2 cell adherence model for further study of the *intimin"** protein.

6/3,AB/25 (Item 22 from file: 144)

DIALOG(R)File 144:Pascal


12272325 PASCAL No.: 95-0502357
The role of the *eaeA"** gene in diarrhea and neurological complications in a gnotobiotic piglet model of *enterohemorrhagic"** Escherichia *coli"** infection
TZIPORI S; GUNZER F; DONNENBERG M S; DE MONTIGNY L; KAPER J B; DONOHUE-ROLFE A
Tufts univ. school veterinary medicine, div. infectious diseases, North Grafton MA 01536, USA
Journal: Infection and immunity, 1995, 63 (9) 3621-3627
Language: English
We reported previously that mutation of the chromosomal gene *eaeA"** from enterohemorragic Escherichia *coli"** (*EHEC"**) serotype O157 :H7 prevented bacterial attachment in vivo. Attachment was restored when the *EHEC"** or *enteropathogenic"** E. *coli"** (EPEC) *eaeA"** gene was introduced into the mutant on a plasmid. In this communication we have compared in gnotobiotic piglets the pathogenicities of wild-type O157 :H7 strain 86-24 and its *eaeA"** mutant UMD619 with those of the two plasmid-complemented strains expressing *Intimin"** SUB O SUB 1 SUB 5 SUB 7 (*EHEC"**) and *Intimin"** SUB O SUB 1 SUB 2 SUB 7 (EPEC). 86-24 colonized the surface and glandular epithelium of the large intestine and induced diarrhea, while UMD619 did not colonize any intestinal site and induced little or no diarrhea. Surprisingly, strain UMD619 expressing *Intimin"** SUB O SUB 1 SUB 2 SUB 7 behaved in pigs more like EPEC than *EHEC"** strains ; it colonized the distal half of the small intestine and the surface of the large intestine, inducing serious diarrhea. In contrast, strain UMD619 expressing *Intimin"** SUB O SUB 1 SUB 5 SUB 7 colonized the colon extremely poorly, inducing little or no diarrhea. While only the two strains causing extensive attachment-86-24 and UMD619 expressing *Intimin"** SUB O SUB 1 SUB 2 SUB 7 -induced diarrhea, neurological symptoms attributed to Shiga-like toxin II occurred equally in all four groups of animals. The intimate bacterial attachment and mucosal damage were not a prerequisite for Shiga-like toxin II translocation from the gut lumen into the circulation. *Intimin"** SUB O SUB 1 SUB 2 SUB 7 appears not only to facilitate intimate attachment to cells but also to influence the site of intestinal colonization and other characteristics of EPEC infection.

6/3,AB/26 (Item 23 from file: 144)
DIALOG(R)File 144:Pascal


12183206 PASCAL No.: 95-0398120
Identification of *EaeA"** protein in the outer membrane of attaching and effacing Escherichia coli O45 from pigs
CHENGRU ZHU; HAREL J; DUMAS F; FAIRBROTHER J M

Univ. Montreal, fac. medecine veterinaire, groupe rech. maladies infectieuses porc, Saint-Hyacinthe PQ J2S 7C6, Canada
Journal: FEMS microbiology letters, 1995, 129 (2-3) 237-242
Language: English
We have previously reported that the production of attaching and effacing lesions by Escherichia *coli"** 045 isolates from pigs is associated with the *eaeA"** (E. *coli"** attaching and effacing) gene. In the present study, expression of the *EaeA"** protein, the *eaeA"** gene product, among swine 045 E. *coli"** isolates was examined. The majority (20/22) of attaching and effacing positive, *eaeA"** SUP + E. *coli"** 045 isolates, but none of ten attaching and effacing negative, *eaeA"** SUP - or *eaeA"** SUP + isolates, expressed a 97-kDa outer membrane protein as revealed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (*SDS"**-*PAGE"**) and Western blot analysis. Amino-terminal amino acid sequencing demonstrated a high homology between this 97-kDa protein of swine E. *coli"** 045 and the *EaeA"** protein (*intimin"**) of human *enteropathogenic"** E. *coli"** and *enterohemorrhagic"** E. *coli"**. In addition, a serological relationship between the *EaeA"** proteins of swine 045, rabbit (RDEC-1) and human (E2348/69) attaching and effacing E. *coli"** strains was observed. Our results indicate an association between expression of the *EaeA"** protein and attaching and efficacing activity among 045 E. *coli"** isolates. The data also suggest an antigenic relatedness of the *EaeA"** proteins of swine, rabbit, and human attaching and effacing E. *coli"**.

6/3,AB/27 (Item 24 from file: 144)
DIALOG(R)File 144:Pascal


12134986 PASCAL No.: 95-0367197
Carbon dioxide regulated secretion of the EaeB protein of *enteropathogenic"** Escherichia *coli"**
HAIGH R; BALDWIN T; KNUTTON S; WILLIAMS P H
Univ. Leicester, dep. microbiology immunology, Leicester LE1 7RH, United Kingdom
Journal: FEMS microbiology letters, 1995, 129 (1) 63-67
Language: English
An *eaeA"** mutant (*intimin"** deficient) of *enteropathogenic"** Escherichia *coli"** stimulated phosphorylation of several host cell proteins, showing that intimate adherence is not required to activate signal transduction pathways in *enteropathogenic"** E. *coli"**-infected cells. Growth of *enteropathogenic"** E. *coli"** in tissue culture medium in 5% CO SUB 2 , in the presence or absence of cultured cells, resulted in the secretion of several bacterial proteins. Two of these, 36 kDa and 20 kDa in size, were expressed at significantly lower levels in air. N-terminal sequencing and analysis of secreted proteins of an eaeB mutant indicated that the 36 kDa secreted protein was EaeB, previously implicated in the stimulation of signalling pathways in *enteropathogenic"** E.

*coli"**-infected cells.

6/3,AB/28 (Item 25 from file: 144)
DIALOG(R)File 144:Pascal


12102725 PASCAL No.: 95-0332204
A plasmid-encoded regulatory region activates chromosomal aeaA expression in *enteropathogenic"** Escherichia *coli"**
GOMEZ-DUARTE O G; KAPER J B
Univ. Maryland school medicine, cent. vaccine development, div. geographic medicine, Baltimore MD 21201, USA
Journal: Infection and immunity, 1995, 63 (5) 1767-1776
Language: English
*Enteropathogenic"** Escherichia *coli"** (EPEC) organisms produce a characteristic histopathology in intestinal epithelial cells called attaching and effacing lesions. The *eaeA"** gene is associated with attaching and effacing lesions and encodes *intimin"**, a 94-kDa outer membrane protein. A 60-MDa plasmid, pMAR2, is essential for full virulence of EPEC strain E2348/69 (O127:H6). We have cloned sequences from pMAR2 that increase expression of the chromosomal *eaeA"** gene as shown by increased alkaline phosphatase activity of an *eaeA"**::TnphoA gene fusion, increased expression of the *intimin"** protein, and increased production of *eaeA"** mRNA. These sequences are called per for plasmid-encoded regulator. pMAR2-cured JPN15 containing cloned per sequences adheres to HEp-2 cells in greater numbers than JPN15 carrying the plasmid vector only. The cloned per sequences contain four open reading frames (ORFs) which have been designated perA through perD. Only perC can by itself activate expression of *eaeA"** ::TnphoA, although the levels of alkaline phosphatase activity seen with this ORF alone are considerably lower than those seen when all four ORFs are present. The molecular sizes of polypeptides predicted from perA, perB, perC, and perD ORFs are 24, 14.8, 10.5, and 9.4 kDa, respectively. The PerA predicted protein shares homology with members of the AraC family of bacterial regulators, but PerB, PerC, and PerD have no striking homology with previously described prokaryotic proteins. Our studies indicate that plasmid-encoded factors regulate the expression of *eaeA"** and possibly genes encoding other outer membrane proteins and may be important for virulence of EPEC

6/3,AB/29 (Item 26 from file: 144)
DIALOG(R)File 144:Pascal


11277267 PASCAL No.: 94-0096533
Expression and characterization of the *eaeA"** gene product of Escherichia coli serotype O157:H7
LOUIE M; DE AZAVEDO J C S; HANDELSMAN M Y C; CLARK C G; ALLY B; DYTOC M;

SHERMAN P; BRUNTON J
Mount Sinai hosp., Samuel Lunenfeld res. inst., Toronto ON M5G 1X5, Canada
Journal: Infection and immunity, 1993, 61 (10) 4085-4092
Language: English
In *enteropathogenic"** Escherichia *coli"**, the *eaeA"** gene produces a 94-kDa outer membrane protein called *intimin"** which has been shown to be necessary but not sufficient to produce the attaching-and-efficacing lesion. The purpose of this study was to characterize the *intimin"** specified by the *eaeA"** allele of the *enterohemorrhagic"** E. *coli"** (*EHEC"** ) serotype 0157:H7 strain CL8 and to determine its role in adherence. The carboxyl-terminal 266 amino acids of the CL8 *intimin"** were expressed as a protein fusion with glutathione S-transferase, which was used to raise antiserum in rabbits. The antiserum reacted in Western immunoblots with a 97-kDa outer membrane protein of *EHEC"** strains of serogroups 05, 026, 0111, and 0157 and *enteropathogenic"** E. *coli"** strains of serogroups 055 and 0127

6/3,AB/30 (Item 1 from file: 266)
DIALOG(R)File 266:FEDRIP


00293372
IDENTIFYING NO.: 5R01AI41325-04 AGENCY CODE: CRISP
NOVEL PROTEINS SECRETED BY E COLI 0157:H7
PRINCIPAL INVESTIGATOR: KAPER, JAMES B
ADDRESS: UNIV OF MARYLAND SCH OF MED 511 W LOMBARD STREET BALTIMORE, MD 21201
PERFORMING ORG.: UNIVERSITY OF MARYLAND BALT PROF SCHOOL, BALTIMORE, MARYLAND
SPONSORING ORG.: NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
FY : 1999
SUMMARY: DESCRIPTION
The major virulence factor of *enterohemorrhagic"** Escherichia *coli"** 0157:H7 is a family of potent cytotoxins known as Shiga toxin, Shiga-like toxin or verocytotoxin. Data from a variety of in vitro, animal, and human studies support the concept that Shiga toxins are crucial for the development of the HUS and hemorrhagic colitis caused by this pathogen. However, there are few data concerning other potential virulence factors expressed by E. *coli"** 0157:H7. Previous work in this laboratory has identified an outer membrane protein known as *intimin"**, encoded by the *eaeA"** gene, which is involved in intestinal colonization by this pathogen. We have recently discovered several bacterial proteins that are secreted into the culture supernatant. These proteins, which are not Shiga toxins and are not produced by non-pathogenic E. *coli"**, produce a strong serum antibody response in patients with HUS. We hypothesize that these proteins play an important role in the pathogenesis of disease due to E. *coli"** 0157:H7. A multi-faceted approach is proposed to investigate these

proteins. The genes encoding these proteins will be cloned and sequenced and the distribution of these genes among other Shiga toxin-producing E. *coli"** will be studied. An ELISA assay will be developed for serodiagnosis and seroepidemiology studies and a large collection of well-characterized patient sera will be screened with this ELISA. Correlations of protein secretion patterns, patient immune response patterns and clinical outcome will be sought to study whether these proteins or the response to them can be used as a marker for clinical outcome. Using purified proteins and isogenic E. *coli"** 0157:H7 strains specifically mutated in genes encoding these proteins, the effect of these proteins on cultured intestinal and renal cells will be studied and potential interactions with Shiga toxin will be examined in these in vitro systems. Isogenic strains mutated in these genes will also be studied in animal models to examine the contribution of these proteins to intestinal and renal manifestations of disease due to E. *coli"** 0157:H7. Together, these studies should yield a more complete picture of disease due to *EHEC"** and offer the possibility of new insights into pathogenesis, improved serodiagnostic methodologies, and identification of potential protective antigens that may be used for development of vaccines or immunotherapeutic agents.

6/3,AB/31 (Item 1 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)


11740916 GENUINE ARTICLE#: 328VN NUMBER OF REFERENCES: 30
TITLE: Crystal structure of *enteropathogenic"** Escherichia *coli"** *intimin"**-receptor complex
AUTHOR(S): Yu L; Frey EA; Pfuetzner RA; Creagh AL; Knoechel DG; Haynes CA; Finlay BB; Strynadka NCJ (REPRINT)
CORPORATE SOURCE: Univ British Columbia, Dept Biochem & Mol Biol, /Vancouver/BC V6T 1Z3/Canada/ (REPRINT); Univ British Columbia, Dept Biochem & Mol Biol, /Vancouver/BC V6T 1Z3/Canada/; Univ British Columbia, Biotechnol Lab, /Vancouver/BC V6T 1Z3/Canada/
PUBLICATION TYPE: JOURNAL
PUBLICATION: NATURE, 2000, V405, N6790 (JUN 29), P1073-1077
PUBLISHER: MACMILLAN PUBLISHERS LTD, PORTERS SOUTH, 4 CRINAN ST, LONDON N1 9XW, ENGLAND
ISSN: 0028-0836
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: *Intimin"** and its *translocated"** *intimin"** receptor ( *Tir"**) are bacterial proteins that mediate adhesion between mammalian cells and attaching and effacing (A/E) pathogens. *Enteropathogenic"** Escherichia *coli"** (EPEC) causes significant paediatric morbidity and mortality world-wide(1). A related A/E pathogen, *enterohaemorrhagic"** E. *coli"** (*EHEC"**; O157:H7) is one of the most important food-borne pathogens in North America, Europe and Japan. A unique and essential feature of A/E bacterial pathogens is the formation of actin-rich

pedestals beneath the intimately adherent bacteria and localized destruction of the intestinal brush border(2). The bacterial outer membrane adhesin, *intimin"**(3), is necessary for the production of the A/E lesion and diarrhoea(4). The A/E bacteria translocate their own receptor for *intimin"**, *Tir"**(5), into the membrane of mammalian cells using the type III secretion system. The translocated *Tir"** triggers additional host signalling events and actin nucleation, which are essential for lesion formation. Here we describe the the crystal structures of an EPEC *intimin"** carboxyterminal fragment alone and in complex with the EPEC *Tir"** *intimin"**-binding domain, giving insight into the molecular mechanisms of adhesion of A/E pathogens.
ISSN: 0028-0836

6/3,AB/32 (Item 2 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)


11732471 GENUINE ARTICLE#: 326AT NUMBER OF REFERENCES: 31
TITLE: Mechanical fractionation reveals structural requirements for *enteropathogenic"** Escherichia *coli"** *Tir"** insertion into host membranes
AUTHOR(S): Gauthier A; de Grado M; Finlay BB (REPRINT)
AUTHOR(S) E-MAIL: bfinlay@unixg.ubc.ca
CORPORATE SOURCE: Univ British Columbia, Biotechnol Lab, /Vancouver/BC V6T 1Z3/Canada/ (REPRINT); Univ British Columbia, Biotechnol Lab, /Vancouver/BC V6T 1Z3/Canada/; Univ British Columbia, Dept Biochem & Mol Biol, /Vancouver/BC V6T 1Z3/Canada/
PUBLICATION TYPE: JOURNAL
PUBLICATION: INFECTION AND IMMUNITY, 2000, V68, N7 (JUL), P4344-4348
PUBLISHER: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA
ISSN: 0019-9567
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: *Enteropathogenic"** Escherichia *coli"** (EPEC) inserts its receptor for intimate adherence (*Tir"**) into host cell membranes by using a type III secretion system, Detergents are frequently used to fractionate infected host cells to investigate bacterial protein delivery into mammalian cells. In this study, we found that the Triton X-100-soluble membrane fraction from EPEC-infected HeLa cells was contaminated with bacterial proteins. We therefore applied a mechanical method of cell lysis and ultracentrifugation to fractionate infected HeLa cells to investigate the biology and biochemistry of *Tir"** delivery and translocation. This method demonstrates that the translocation of *Tir"** into the host cell membrane requires its transmembrane domains, but not tyrosine phosphorylation or binding to *Tir"**'s ligand, *intimin"**.
ISSN: 0019-9567

6/3,AB/33 (Item 3 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)


11716939 GENUINE ARTICLE#: 324AT NUMBER OF REFERENCES: 44
TITLE: *Intimin"** from *enteropathogenic"** Escherichia *coli"** mediates remodelling of the eukaryotic cell surface
AUTHOR(S): Phillips AD (REPRINT); Giron J; Hicks S; Dougan G; Frankel G
AUTHOR(S) E-MAIL: adphill@rfhsm.ac.uk
CORPORATE SOURCE: Univ London, Royal Free Hosp, /London NW3 2QG//England/ (REPRINT); Univ London, Royal Free Hosp, /London NW3 2QG//England/; Univ Autonoma Puebla, Ctr Invest Microbiol Benemerita, /Puebla 72570//Mexico/; Univ London Imperial Coll Sci Technol & Med, Dept Biochem, /London SW7 2AZ//England/
PUBLICATION TYPE: JOURNAL
PUBLICATION: MICROBIOLOGY-UK, 2000, V146, ,6 (JUN), P1333-1344
PUBLISHER: SOC GENERAL MICROBIOLOGY, MARLBOROUGH HOUSE, BASINGSTOKE RD, SPENCERS WOODS, READING RG7 1AE, BERKS, ENGLAND
ISSN: 1350-0872
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: Adhesion to cultured epithelial cells by enteropathogenic Escherichia coil (EPEC) is associated with extensive rearrangement of the host cell cytoskeleton. Evidence has been presented that EPEC adhesion is associated with activation of signal transduction pathways leading to production of a characteristic histopathological feature known as the attaching and effacing (A/E) lesion. A/E lesion formation requires *intimin"**, an EPEC adhesion molecule and several EPEC secreted proteins (EspA, B, D and *Tir"**) involved in cell signalling and protein translocation. In this study it is shown that HEp-2 cells respond during the early stages of infection with two wild-type EPEC strains (B171 and E2348/69) by producing microvillus-like processes (MLP) at the site of initial bacterial adherence. *Intimin"** appears to play a key role in MLP elongation. At later stages of infection with these wild-type EPEC strains, when A/E lesions have formed, the MLP were reduced in number and length to appear as at time zero, and the cell surface in the vicinity of bacterial clusters appeared unaffected. In contrast, infection with EspA- or EspB-negative, but *intimin"** -positive, EPEC strains (UMD872 and UMD864, respectively) resulted in enhanced MLP proliferation and formation of 'cage-like' structures engulfing the bacteria. Inoculating HEp-2 cells with *intimin"**-coated latex spheres induced similar 'cage-like' structures. Caco-2 cells did not show *intimin"**-induced microvillus elongation in response to EPEC infection, although microvillus effacement and reduction in number occurred. Similar phenomena appeared on B171 and E2348/69 infection of paediatric intestine using in vitro organ culture, i.e. elongated microvilli were seen in association with small colonies and at the periphery of large localized colonies, along with evidence of microvillus breakdown and debris in the colony centre. These results

show that *intimin"** activates signal transduction pathways involved in the remodelling of the eukaryotic cell surface, probably via binding to a receptor encoded by the host cell.
ISSN: 1350-0872

6/3,AB/34 (Item 4 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)


11709866 GENUINE ARTICLE#: 322ZH NUMBER OF REFERENCES: 59
TITLE: Structural basis for recognition of the *translocated"** *intimin"** receptor (*Tir"**) by *intimin"** from *enteropathogenic"** Escherichia *coli"**
AUTHOR(S): Batchelor M; Prasannan S; Daniell S; Reece S; Connerton I; Bloomberg G; Dougan G; Frankel G (REPRINT); Matthews S
AUTHOR(S) E-MAIL: s.j.matthews@ic.ac.uk
CORPORATE SOURCE: Univ London Imperial Coll Sci Technol & Med, Dept Biochem, /London SW7 2AZ//England/ (REPRINT); Univ London Imperial Coll Sci Technol & Med, Dept Biochem, /London SW7 2AZ//England/; Univ London Imperial Coll Sci Technol & Med, Ctr Struct Biol, /London SW7 2AZ//England/; Univ Nottingham, Div Food Sci, /Loughborough LE12 5RD/Leics/England/; Univ Bristol, Dept Biochem, /Bristol BS8 1TD/Avon/England/
PUBLICATION TYPE: JOURNAL
PUBLICATION: EMBO JOURNAL, 2000, V19, N11 (JUN 1), P2452-2464
PUBLISHER: OXFORD UNIV PRESS, GREAT CLARENDON ST, OXFORD OX2 6DP, ENGLAND
ISSN: 0261-4189
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: *Intimin"** is a bacterial adhesion molecule involved in intimate attachment of *enteropathogenic"** and *enterohaemorrhagic"** Escherichia *coli"** to mammalian host cells. *Intimin"** targets the *translocated"** *intimin"** receptor (*Tir"**), which is exported by the bacteria and integrated into the host cell plasma membrane. In this study we localized the *Tir"**-binding region of *intimin"** to the C-terminal 190 amino acids (Int190), We have also determined the region's high-resolution solution structure, which comprises an immunoglobulin domain that is intimately coupled to a novel C-type lectin domain, This fragment, which is necessary and sufficient for *Tir"** interaction, defines a new super domain in *intimin"** that exhibits striking structural similarity to the integrin-binding domain of the Yersinia invasin and C-type lectin families, The extracellular portion of *intimin"** comprises an articulated rod of immunoglobulin domains extending from the bacterium surface, conveying a highly accessible 'adhesive tip' to the target cell. The interpretation of NMR-titration and mutagenesis data has enabled us to identify, for the first time, the binding site for *Tir"**, which is located at the extremity of the Int190 moiety.
ISSN: 0261-4189

6/3,AB/35 (Item 5 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)


11628803 GENUINE ARTICLE#: 315HH NUMBER OF REFERENCES: 136
TITLE: Virulence factors of Escherichia coli O157 and other Shiga toxin-producing E-coli
AUTHOR(S): Law D (REPRINT)
CORPORATE SOURCE: IDG UK, Topley House,52 Wash Lane/Bury BL9 6AU//England/ (REPRINT); Hyder Environm, /Runcorn/Cheshire/England/
PUBLICATION TYPE: JOURNAL
PUBLICATION: JOURNAL OF APPLIED MICROBIOLOGY, 2000, V88, N5 (MAY), P729-745
PUBLISHER: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 0NE, OXON, ENGLAND
ISSN: 1364-5072
LANGUAGE: English DOCUMENT TYPE: REVIEW
ABSTRACT: Shiga toxin-producing Escherichia roll O157 are important *enteropathogens"** causing outbreaks of haemorrhagic colitis and haemolytic uraemic syndrome. Strains of E. *coli"** belonging to other serogroups also produce Shiga toxins but are less frequently isolated from cases of diarrhoeal illness despite humans having greater exposure to these organisms in food and the environment. It is generally considered that E. *coli"** O157 is more virulent than these other Shiga toxin-producing E. *coli"**. The question of which factors make toxigenic E. *coli"** O157 more virulent is unanswered. In this review the various virulence properties of E. *coli"** O157 and their incidence in non-O157 Shiga toxin-producing E. *coli"** are described. The most important factors for E. *coli"** O157 are the production of Shiga toxin 2 and the adhesin *intimin"**. The role of some of the other virulence factors, such as enterohaemolysin, a serine protease (EspP) and a catalase/peroxidase (Katp), in infection may be low. Uncharacterized virulence properties such as a clostridial-like toxin and haemoglobin uptake require further study and it is likely that other virulence properties remain to be discovered.
ISSN: 1364-5072

6/3,AB/36 (Item 6 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)


11387136 GENUINE ARTICLE#: BP55D NUMBER OF REFERENCES: 29
TITLE: The locus for enterocyte effacement (LEE) of *enteropathogenic"** Escherichia *coli"** (EPEC) from dogs and cats
AUTHOR(S): Goffaux F (REPRINT); China B; Janssen L; Pirson V; Mainil J; Paul PS; Francis DH
CORPORATE SOURCE: Univ Liege, Bacteriol Lab, Sart Tilman B43A/B-4000

Liege//Belgium/ (REPRINT); Univ Liege, Bacteriol Lab, /B-4000 Liege//Belgium/
PUBLICATION TYPE: BOOK IN SERIES
PUBLICATION: MECHANISMS IN THE PATHOGENESIS OF ENTERIC DISEASES 2, 1999, V 473, P129-136
BOOK SERIES TITLE: ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY
PUBLISHER: KLUWER ACADEMIC / PLENUM PUBL, 233 SPRING ST, NEW YORK, NY 10013 USA
ISBN: 0-306-46214-1 LIBRARY OF CONGRESS ID: 99-048628
ISSN: 0065-2598
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: *Enteropathogenic"** Escherichia *coli"** (EPEC) produce attaching and effacing lesions. The genes responsible for this lesion are clustered on the chromosome forming a 35.5 kilobase pathogenesis island called LEE. The LEE was identified: characterized and completely sequenced from the human EPEC strain E2348/69. The LEE carries genes coding for: a type III secretion system (genes esc and sep), the *translocated"** *intimin"** receptor (gene *tir"**), the outer membrane protein *intimin"** (gene eae) and the E. *coli"** secreted proteins EspA, EspB, and EspD (genes esp). In addition to man and farm animals, EPEC are also isolated from dogs and cats. We studied structurally and functionally the LEE of dog and cat EPEC. First, we used four probes scattered along the LEE to identify the presence of a LEE in canine and feline EPEC isolates. Second, by PCR, we checked the presence of genes homologous to ene, sep, esp, and *tir"** genes in these strains. Third, since the four types of ene and *tir"** genes were described, we developed a multiplex PCR in order to determine the type of eae and *tir"** genes present in each strain. Fourth, we determined by PCR the site of the LEE insertion on the chromosome. Fifth, we tested several of the canine EPEC in their capacity to induce attaching and effacing lesions in the rabbit intestinal loop assay. We can conclude from this study: first, that the a LEE-like structure is present in all tested strains and that it contains genes homologous to esp, sep, *tir"**, and eae genes; second, that there is some preferential associations between the type of eae gene and the type of *tir"** gene present in a strain: third, that the majority of the tested strains contained a LEE located elsewhere on the chromosome in comparison to the human EPEC strain E2348/69: and fourth that dog EPEC were able to induce attaching and effacing lesions in rabbit ileal loop assay.
ISSN: 0065-2598

6/3,AB/37 (Item 7 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)


11236777 GENUINE ARTICLE#: 271PR NUMBER OF REFERENCES: 21
TITLE: Human colostrum and serum contain antibodies reactive to the

*intimin"**-binding region of the *enteropathogenic"** Escherichia *coli"** *translocated"** *intimin"** receptor

AUTHOR(S): Sanches MI; Keller R; Hartland EL; Figueiredo DMM; Batchelor M; Martinez MB; Dougan G; Careiro-Sampaio MMS; Frankel G (REPRINT); Trabulsi LR

CORPORATE SOURCE: Univ London Imperial Coll Sci Technol & Med, Dept Biochem, /London SW7 2AZ//England/ (REPRINT); Univ London Imperial Coll Sci Technol & Med, Dept Biochem, /London SW7 2AZ//England/; Univ Sao Paulo, Dept Immunol, /Sao Paulo//Brazil/; Univ Sao Paulo, Dept Anal Clin & Toxicol, /Sao Paulo//Brazil/

PUBLICATION TYPE: JOURNAL

PUBLICATION: JOURNAL OF PEDIATRIC GASTROENTEROLOGY AND NUTRITION, 2000, V30, N1 (JAN), P73-77

PUBLISHER: LIPPINCOTT WILLIAMS & WILKINS, 530 WALNUT ST, PHILADELPHIA, PA 19106-3621 USA

ISSN: 0277-2116

LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: Background: In Brazil, *enteropathogenic"** Escherichia *coli"** (EPEC) diarrhoea is endemic in young infants. A characteristic feature of EPEC adhesion to host cells is intimate attachment leading to the formation of distinctive "attaching and effacing" (A/E) lesions on mammalian cells. Two genes directly involved in intimate adhesion, ene and *tir"**, encode the adhesion molecule *intimin"** and its translocated receptor *Tir"**, respectively. The *intimin"**-binding domain of *Tir"** was recently mapped to the middle part of the polypeptide (*Tir"**-M), and the amino (*Tir"**-N) and carboxy (*Tir"**-C) termini were found to be located within infected host cells. Recently, it was shown that colostrum samples from mothers living in Sao Paulo contain IgA-class antibodies reactive with a number of proteins associated with EPEC virulence. It has also been shown that patients infected with verocytotoxin-producing E. *coli"** O157 can produce antibodies to *Tir"**. In the current study antibody responses to the different *Tir"** domains were analyzed in sera and colostrum samples collected in an EPEC-endemic area of Brazil.

Methods: Recombinant *Tir"**, *Tir"**-N, *Tir"**-M, and *Tir"**-C were expressed as His-tagged protein in E. coli BL21a and purified on nickel columns. Western blot analysis was used to investigate colostrum IgA- and serum IgG-class antibodies reactive with the *Tir"** fragments.

Results: Anti-*Tir"** IgG antibodies were detected in the serum of children, with (63%) or without (50%) diarrhoea, Anti-*Tir"** IgA-class antibodies were detected in all the colostrum pools tested. With the use of both serum IgG- and colostrum IgA-class antibodies, an immunodominant domain of the *Tir"**-polypeptide, *Tir"** M, was identified.

Conclusion: The *intimin"**-binding region of *Tir"** (*Tir"**-M) is the immunodominant region of the polypeptide in humans. Both serum

IgG-class and colostrum IgA-class antibodies reacted predominantly with the *Tir"**-M domain. (C) 2000 Lippincott Williams & Wilkins, Inc.
ISSN: 0277-2116

6/3,AB/38 (Item 8 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)


11216985 GENUINE ARTICLE#: 268UL NUMBER OF REFERENCES: 27
TITLE: Hierarchy in the expression of the locus of enterocyte effacement genes of *enteropathogenic"** Escherichia *coli"**
AUTHOR(S): Friedberg D; Umanski T; Fang YA; Rosenshine I (REPRINT)
AUTHOR(S) E-MAIL: ilanro@cc.huji.ac.il
CORPORATE SOURCE: Hebrew Univ Jerusalem, Dept Mol Genet, POB 12272/IL-91120 Jerusalem//Israel/ (REPRINT); Hebrew Univ Jerusalem, Dept Mol Genet, /IL-91120 Jerusalem//Israel/; Hebrew Univ Jerusalem, Dept Biotechnol, /IL-91120 Jerusalem//Israel/
PUBLICATION TYPE: JOURNAL
PUBLICATION: MOLECULAR MICROBIOLOGY, 1999, V34, N5 (DEC), P941-952
PUBLISHER: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 0NE, OXON, ENGLAND
ISSN: 0950-382X
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: *Enteropathogenic"** Escherichia *coli"** (EPEC) elicit changes in host cell morphology and cause actin rearrangement, a phenotype that has commonly been referred to as attaching/effacing (AE) lesions. The ability of EPEC to induce AE lesions is dependent upon a type III protein secretion/translocation system that is encoded by genes clustered in a 35.6 kb DNA segment, named the locus of enterocyte effacement (LEE). We used transcriptional fusions between the green fluorescent protein (gfp) reporter gene and LEE genes rorf2, orf3, orf5, escJ, escV and eae, together with immunoblot analysis with antibodies against *Tir"**, *intimin"**, EspB and EspF, to analyse the genetic regulation of the LEE. The expression of all these LEE genes was strictly dependent upon the presence of a functional integration host factor (IHF). IHF binds specifically upstream from the ler (orf1) promoter and appears to activate expression of ler, orf3, orf5 and rorf2 directly. The ler-encoded Ler protein was involved in activating the expression of escJ, escV, *tir"**, eae, espB and espF. Expression of both IHF and Ler was needed to elicit actin rearrangement associated with AE lesions. In conclusion, IHF directly activates the expression of the ler and rorf2 transcriptional units, and Ler in turn mediates the expression of the other LEE genes.
ISSN: 0950-382X

6/3,AB/39 (Item 9 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

11203169 GENUINE ARTICLE#: 267TM NUMBER OF REFERENCES: 41
TITLE: Quorum sensing controls expression of the type III secretion gene transcription and protein secretion in *enterohemorrhagic"** and *enteropathogenic"** Escherichia *coli"**
AUTHOR(S): Sperandio V; Mellies JL; Nguyen W; Shin S; Kaper JB (REPRINT)
AUTHOR(S) E-MAIL: jkaper@umaryland.edu
CORPORATE SOURCE: Univ Maryland, Ctr Vaccine Dev, 685 W Baltimore St/Baltimore//MD/21201 (REPRINT); Univ Maryland, Ctr Vaccine Dev, /Baltimore//MD/21201; Univ Maryland, Dept Microbiol & Immunol, /Baltimore//MD/21201
PUBLICATION TYPE: JOURNAL
PUBLICATION: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, 1999, V96, N26 (DEC 21), P15196-15201
PUBLISHER: NATL ACAD SCIENCES, 2101 CONSTITUTION AVE NW, WASHINGTON, DC 20418 USA
ISSN: 0027-8424
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: *Enterohemorrhagic"** Escherichia *coli"** O157:H7 and *enteropathogenic"** E. *coli"** cause a characteristic histopathology in intestinal cells known as attaching and effacing. The attaching and effacing lesion is encoded by the Locus of Enterocyte Effacement (LEE) pathogenicity island, which encodes a type III secretion system, the *intimin"** intestinal colonization factor, and the *translocated"** *intimin"** receptor protein that is translocated from the bacterium to the host epithelial cells. Using lacZ reporter gene fusions, we show that expression of the LEE operons encoding the type III secretion system, *translocated"** *intimin"** receptor, and *intimin"** is regulated by quorum sensing in both *enterohemorrhagic"**: E. *coli"** and *enteropathogenic"** E. *coli"**, The luxS gene recently shown to be responsible for production of autoinducer in the Vibrio harveyi and E, *coli"** quorum-sensing systems is responsible for regulation of the LEE operons, as shown by the mutation and complementation of the luxS gene. Regulation of intestinal colonization factors by quorum sensing could play an important role in the pathogenesis of disease caused by these organisms. These results suggest that intestinal colonization by E. *coli"** O157:H7, which has an unusually low infectious dose, could be induced by quorum sensing of signals produced by nonpathogenic E. *coli"** of the normal intestinal flora.
ISSN: 0027-8424

6/3,AB/40 (Item 10 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)


11013552 GENUINE ARTICLE#: 245EX NUMBER OF REFERENCES: 60
TITLE: The *Tir"**-binding region of *enterohaemorrhagic"** Escherichia

*coli"** *intimin"** is sufficient to trigger actin condensation after bacterial-induced host cell signalling
AUTHOR(S): Liu H; Magoun L; Luperchio S; Schauer DB; Leong JM (REPRINT)
AUTHOR(S) E-MAIL: john.leong@umassmed.edu
CORPORATE SOURCE: Univ Massachusetts, Dept Mol Genet & Microbiol, 55 Lake Ave N/Worcester//MA/01655 (REPRINT); Univ Massachusetts, Dept Mol Genet & Microbiol, /Worcester//MA/01655; MIT, Dept Bioengn & Environm Hlth, /Cambridge//MA/02139; MIT, Div Comparat Med, /Cambridge//MA/02139
PUBLICATION TYPE: JOURNAL
PUBLICATION: MOLECULAR MICROBIOLOGY, 1999, V34, N1 (OCT), P67-81
PUBLISHER: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 0NE, OXON, ENGLAND
ISSN: 0950-382X
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: *Enterohaemorrhagic"** Escherichia *coli"** (*EHEC"**) has emerged as an important agent of diarrhoeal disease. Attachment to host cells, an essential step during intestinal colonization by *EHEC"**, is associated with the formation of a highly organized cytoskeletal structure containing filamentous actin, termed an attaching and effacing (A/E) lesion, directly beneath bound bacteria. The outer membrane protein *intimin"** is required for the formation of this structure, as is *Tir"**, a bacterial protein that is translocated into the host cell and is thought to function as a receptor for *intimin"**, To understand *intimin"** function better, we fused *EHEC"** *intimin"** to a homologous protein, Yersinia pseudotuberculosis invasin, or to maltose-binding protein. The N-terminal 539 amino acids of *intimin"** were sufficient to promote outer membrane localization of the C-terminus of invasin and, conversely, the N-terminal 489 amino acids of invasin were sufficient to promote the localization of the C-terminus of *intimin"**, The C-terminal 181 residues of *intimin"** were sufficient to bind mammalian cells that had been preinfected with an *enteropathogenic"** E. *coli"** strain that expresses *Tir"** but not *intimin"**. Binding of *intimin"** derivatives to preinfected cells correlated with binding to recombinant *Tir"** protein. Finally, the 181-residue minimal *Tir"**-binding region of *intimin"**, when purified and immobilized on latex beads, was sufficient to trigger A/E lesions on preinfected mammalian cells.
ISSN: 0950-382X

6/3,AB/41 (Item 11 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)


10970744 GENUINE ARTICLE#: 240GP NUMBER OF REFERENCES: 47
TITLE: Identification of CesT, a chaperone for the type III secretion of *Tir"** in *enteropathogenic"** Escherichia *coli"**
AUTHOR(S): Elliott SJ; Hutcheson SW; Dubois MS; Mellies JL; Wainwright LA; Batchelor M; Frankel G; Knutton S; Kaper JB (REPRINT)
Searcher : Shears 308-4994

AUTHOR(S) E-MAIL: jkaper@umaryland.edu
CORPORATE SOURCE: Univ Maryland, Ctr Vaccine Dev, 685 W Baltimore St/Baltimore//MD/21201 (REPRINT); Univ Maryland, Ctr Vaccine Dev, /Baltimore//MD/21201; Univ Maryland, Dept Microbiol & Immunol, /Baltimore//MD/21201; Univ Maryland, Dept Mol Genet & Cell Biol, /College Pk//MD/20742; Univ London Imperial Coll Sci Technol & Med, Dept Biochem, /London SW7 2AZ//England/; Univ Birmingham, Inst Child Hlth, /Birmingham B16 8ET/W Midlands/England/
PUBLICATION TYPE: JOURNAL
PUBLICATION: MOLECULAR MICROBIOLOGY, 1999, V33, N6 (SEP), P1176-1189
PUBLISHER: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 0NE, OXON, ENGLAND
ISSN: 0950-382X
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: The locus of enterocyte effacement of *enteropathogenic"** Escherichia *coli"** encodes a type III secretion system, an outer membrane protein adhesin (*intimin"**, the product of eae) and *Tir"**, a translocated protein that becomes a host cell receptor for *intimin"**. Many type III secreted proteins require chaperones, which function to stabilize proteins, prevent inappropriate protein-protein interactions and aid in secretion. An open reading frame located between fir and eae, previously named orfU, was predicted to encode a protein with partial similarity to the Yersinia SycH chaperone. We examined the potential of the orfU gene product to serve as a chaperone for *Tir"**. The orfU gene encoded a 15 kDa cytoplasmic protein that specifically interacted with *Tir"** as demonstrated by the yeast two-hybrid assay, column binding and coimmunoprecipitation experiments. An orfU mutant was defective in attaching-effacing lesion formation and *Tir"** secretion, but was unaffected in expression of other virulence factors. OrfU:appeared to stabilize *Tir"** levels in the cytoplasm, but was not absolutely necessary for secretion of *Tir"**. Based upon the physical similarities, phenotypic characteristics and the demonstrated interaction with *Tir"**, orfU is redesignated as cesT for the chaperone for E. *coli"** secretion of *Tir"**.
ISSN: 0950-382X

6/3,AB/42 (Item 12 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)


10970743 GENUINE ARTICLE#: 240GP NUMBER OF REFERENCES: 57
TITLE: *Enteropathogenic"** Escherichia *coli"** *translocated"** *intimin"** receptor, *Tir"**, requires a specific chaperone for stable secretion
AUTHOR(S): Abe A; de Grado M; Pfuetzner RA; Sanchez-SanMartin C; DeVinney R ; Puente JL; Strynadka NCJ; Finlay BB (REPRINT)
AUTHOR(S) E-MAIL: bfinlay@unixg.ubc.ca
CORPORATE SOURCE: Univ British Columbia, Biotechnol Lab, Room 237,Wesbrook

Bldg,6174 Univ Blvd/Vancouver/BC V6T 1Z3/Canada/ (REPRINT); Univ British Columbia, Biotechnol Lab, /Vancouver/BC V6T 1Z3/Canada/; Univ British Columbia, Dept Biochem & Mol Biol, /Vancouver/BC V6T 1Z3/Canada/; Kitasato Inst, Minato Ku, /Tokyo 108//Japan/; Univ Nacl Autonoma Mexico, Dept Mol Microbiol, /Cuernavaca 62250/Morelos/Mexico/
PUBLICATION TYPE: JOURNAL
PUBLICATION: MOLECULAR MICROBIOLOGY, 1999, V33, N6 (SEP), P1162-1175
PUBLISHER: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 0NE, OXON, ENGLAND
ISSN: 0950-382X
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: *Enteropathogenic"** Escherichia *coli"** (EPEC) secretes several Esps (E. *coli"**-secreted proteins) that are required for full virulence. Insertion of the bacterial protein *Tir"** into the host epithelial cell membrane is facilitated by a type III secretion apparatus, and at least EspA and EspB are required for *Tir"** translocation. An EPEC outer membrane protein, *intimin"**, interacts with *Tir"** on the host membrane to establish intimate attachment and formation of a pedestal-like structure. In this study, we identified a *Tir"** chaperone, CesT, whose gene is located between *tir"** and eae (which encodes *intimin"**). A mutation in cesT abolished *Tir"** secretion into culture supernatants and significantly decreased the amount of *Tir"** in the bacterial cytoplasm. In contrast, this mutation did not affect the secretion of the Esp proteins. The level of *tir"** mRNA was not affected by the cesT mutation, indicating that CesT acts at the post-transcriptional level. The cesT mutant could not induce host cytoskeletal rearrangements, and displayed the same phenotype as the fir mutant. Gel overlay and GST pulldown assays demonstrated that CesT specifically interacts with *Tir"**, but not with other Esp proteins. Furthermore, by using a series of *Tir"** deletion derivatives, we determined that the CesT binding domain is located within the first 100 amino-terminal residues of *Tir"**, and that the pool of *Tir"** in the bacterial cytoplasm was greatly reduced when this domain was disrupted. Interestingly, this domain was not sufficient for *Tir"** secretion, and at least the first 200 residues of *Tir"** were required for efficient secretion. Gel filtration studies showed that *Tir"**-CesT forms a large multimeric complex. Collectively, these results indicate that CesT is a *Tir"** chaperone that may act as an anti-degradation factor by specifically binding to its amino-terminus, forming a multimeric stabilized complex.
ISSN: 0950-382X

6/3,AB/43 (Item 13 from file: 440)


10830040 GENUINE ARTICLE#: 226BD NUMBER OF REFERENCES: 49
TITLE: Semi-automated fluorogenic PCR assays (TaqMan) for vapid detection

of Escherichia coli O157 : H7 and other Shiga toxigenic E. coli
AUTHOR(S): Sharma VK (REPRINT); Dean-Nystrom EA; Casey TA
AUTHOR(S) E-MAIL: vsharma@nadc.ars.usda.gov
CORPORATE SOURCE: USDA ARS, Enter Dis & Food Safety Res Unit, POB 70/Ames//IA/50010 (REPRINT); USDA ARS, Enter Dis & Food Safety Res Unit, /Ames//IA/50010
PUBLICATION TYPE: JOURNAL
PUBLICATION: MOLECULAR AND CELLULAR PROBES, 1999, V13, N4 (AUG), P291-302
PUBLISHER: ACADEMIC PRESS LTD, 24-28 OVAL RD, LONDON NW1 7DX, ENGLAND
ISSN: 0890-8508
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: Semi-automated detection of *Enterohaemorrhagic"** Escherichia *coli"** (*EHEC"**) O157:H7 and non-O157:H7 Shiga toxin-producing E. *coli"** (STEC) was achieved using fluorogenic polymerase chain reaction (PCR). These PCR assays were designed to amplify 80, 120 and 150 bp regions of virulence genes stx1, stx2 and *eaeA"**, respectively, using specific primers. The fluorogenic probes were used for specific detection of amplified products of the stx1 and stx2 genes of STEC, and the *eaeA"** gene of *EHEC"** O157:H7. For multiplex PCR assay, the three sets of primers and fluorogenic probes were included in one reaction to simultaneously amplify and detect any of the three targeted virulence genes. In non-multiplex PCR assay, each of the three virulence genes was amplified and detected in independent reactions. The specificity of these assays was evaluated using suspensions of STEC and other bacterial species lacking stx1, stx2 and *eaeA"**. The multiplex assay detected all STEC harbouring any combination of th ree virulence genes. Three non-multiplex PCR reactions identified types of Shiga toxin genes carried by a STEC and identified STEC as either *EHEC"** O157:H7 or non-O157:H7 STEC. Sensitivity limits of these assays in beef and faeces inoculated with *EHEC"** O157:H7 were 5.8 to 580 cfu and 1.2 to 1200 cfu, respectively. These assays can be completed within 8-10 h when performed simultaneously or within 13 h if the multiplex assay is used as an initial screen for detecting STEC and the non-multiplex assay is used for subsequent detection of stx1 and stx2 of STEC and *eaeA"** of *EHEC"** O157:H7.
ISSN: 0890-8508

6/3,AB/44 (Item 14 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)


10665713 GENUINE ARTICLE#: 209MM NUMBER OF REFERENCES: 122
TITLE: *Enteropathogenic"** Escherichia *coli"**: a pathogen that inserts its own receptor into host cells
AUTHOR(S): DeVinney R; Gauthier A; Abe A; Finlay BB (REPRINT)
AUTHOR(S) E-MAIL: bfinlay@unixg.ubc.ca
CORPORATE SOURCE: Univ British Columbia, Biotechnol Lab, /Vancouver/BC V6T 1Z4/Canada/ (REPRINT); Univ British Columbia, Biotechnol Lab,

/Vancouver/BC V6T 1Z4/Canada/; Kitasato Inst, Minato Ku, /Tokyo 108//Japan/
PUBLICATION TYPE: JOURNAL
PUBLICATION: CELLULAR AND MOLECULAR LIFE SCIENCES, 1999, V55, N6-7 (JUN), P 961-976
PUBLISHER: BIRKHAUSER VERLAG AG, VIADUKSTRASSE 40-44, PO BOX 133, CH-4010 BASEL, SWITZERLAND
ISSN: 1420-682X
LANGUAGE: English DOCUMENT TYPE: REVIEW
ABSTRACT: *Enteropathogenic"** Escherichia *coli"** (EPEC) is a major cause of infant diarrhea, killing hundreds of thousands of children per year worldwide. Intimate attachment to the host cell leading to the formation of actin-rich pedestals beneath the adhering bacteria is an essential feature of EPEC pathogenesis. EPEC attaches to host cells via the outer membrane adhesin, *intimin"**. It was recently shown that EPEC inserts its own receptor for intimate adherence, *Tir"** ( *translocated"** *intimin"** receptor) into the host cell membrane. The focus of this review is on the discovery and characterization of this novel receptor, and our current understanding of its role in pedestal formation. Gram-negative bacterial secretion systems, including type III secretion systems, are reviewed and discussed in the context of *Tir"** delivery into the host cell membrane. The relationship and relevance of in vitro models compared to the actual in vivo situation is essential to understanding disease. We have critically reviewed the use of animal models in studying EPEC infection. Elucidating the function of *Tir"** will contribute to our understanding of how EPEC mediates disease.
ISSN: 1420-682X

6/3,AB/45 (Item 15 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)


10475977 GENUINE ARTICLE#: 187BW NUMBER OF REFERENCES: 26
TITLE: Binding of *intimin"** from *enteropathogenic"** Escherichia *coli"** to *Tir"** and to host cells
AUTHOR(S): Hartland EL; Batchelor M; Delahay RM; Hale C; Matthews S; Dougan G; Knutton S; Connerton I; Frankel G (REPRINT)
AUTHOR(S) E-MAIL: g.frankel@ic.ac.uk
CORPORATE SOURCE: Univ London Imperial Coll Sci Technol & Med, Dept Biochem, /London SW7 2AZ//England/ (REPRINT); Univ London Imperial Coll Sci Technol & Med, Dept Biochem, /London SW7 2AZ//England/; Univ London Imperial Coll Sci Technol & Med, Ctr Struct Biol, /London SW7 2AZ//England/; Univ Birmingham, Inst Child Hlth, /Birmingham B4 6NH/W Midlands/England/; Inst Food Res, Reading Lab, /Reading RG6 6BZ/Berks/England/; Univ Nottingham, Div Food Sci, /Loughborough LE12 5RD/Leics/England/
PUBLICATION TYPE: JOURNAL

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3/3,AB/1 (Item 1 from file: 144)
DIALOG(R)File 144:Pascal


12183206 PASCAL No.: 95-0398120
Identification of *EaeA"** protein in the outer membrane of attaching and effacing Escherichia coli O45 from pigs
CHENGRU ZHU; HAREL J; DUMAS F; FAIRBROTHER J M
Univ. Montreal, fac. medecine veterinaire, groupe rech. maladies infectieuses porc, Saint-Hyacinthe PQ J2S 7C6, Canada
Journal: FEMS microbiology letters, 1995, 129 (2-3) 237-242
Language: English
We have previously reported that the production of attaching and effacing lesions by Escherichia coli 045 isolates from pigs is associated with the *eaeA"** (E. coli attaching and effacing) gene. In the present study, expression of the *EaeA"** protein, the *eaeA"** gene product, among swine 045 E. coli isolates was examined. The majority (20/22) of attaching and effacing positive, *eaeA"** SUP + E. coli 045 isolates, but none of ten attaching and effacing negative, *eaeA"** SUP - or *eaeA"** SUP + isolates, expressed a 97-kDa outer membrane protein as revealed by *sodium"** *dodecyl"** sulfate-polyacrylamide gel *electrophoresis"** (*SDS"**-*PAGE"** ) and Western blot analysis. Amino-terminal amino acid sequencing demonstrated a high homology between this 97-kDa protein of swine E. coli 045 and the *EaeA"** protein (intimin) of human enteropathogenic E. coli and enterohemorrhagic E. coli. In addition, a serological relationship between the *EaeA"** proteins of swine 045, rabbit (RDEC-1) and human (E2348/69) attaching and effacing E. coli strains was observed. Our results indicate an association between expression of the *EaeA"** protein and attaching and efficacing activity among 045 E. coli isolates. The data also suggest an antigenic relatedness of the *EaeA"** proteins of swine, rabbit, and human attaching and effacing E. coli.

3/3,AB/2 (Item 1 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)


11235993 GENUINE ARTICLE#: 271RC NUMBER OF REFERENCES: 16
TITLE: Antibody response of patients infected with verocytotoxin-producing Escherichia coli to protein antigens encoded on the LEE locus
AUTHOR(S): Jenkins C; Chart H (REPRINT); Smith HR; Hartland EL; Batchelor M ; Delahay RM; Dougan G; Frankel G
AUTHOR(S) E-MAIL: hchart@phls.co.uk
CORPORATE SOURCE: Cent Publ Hlth Lab, Lab Enter Pathogens, 61 Colindale Ave/London NW9 5HT//England/ (REPRINT); Cent Publ Hlth Lab, Lab Enter Pathogens, /London NW9 5HT//England/; Univ London Sch Pharm, Dept Biochem, /London SW7 2AY//England/

PUBLICATION TYPE: JOURNAL
PUBLICATION: JOURNAL OF MEDICAL MICROBIOLOGY, 2000, V49, N1 (JAN), P97-101
PUBLISHER: LIPPINCOTT WILLIAMS & WILKINS, 530 WALNUT ST, PHILADELPHIA, PA 19106-3621 USA
ISSN: 0022-2615
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: Sera from patients infected with verocytotoxin-producing Escherichia coli (VTEC) O157, from patients with antibodies to E. coli O157 lipopolysaccharide (LPS) and from healthy controls were examined for antibodies to proteins involved in expressing the attaching and effacing phenotype, After *SDS"**-*PAGE"**, purified recombinant intimin, EspA-filament structural protein, translocated protein EspB and three separate domains of the *translocated"** *intimin"** receptor (*Tir"**) were tested for reaction with patients' sera by immunoblotting, An ELISA was also used to detect antibodies to intimin in sera from E, coli O157 LPS antibody-positive individuals. Seven of nine culture-positive patients and one control patient had antibodies to EspA, Five of these patients and two controls had serum antibodies to the intimin-binding region of Tit, whereas none of the sera contained antibodies binding to either of the intracellular domains of *Tir"**, By immunoblotting, 10 of 14 culture-positive patients had antibodies to the conserved region of intimin, eight of whom were infected with E, coli O157 phage type 2, Thirty-six of 60 sera from culture-negative but E coli O157 LPS antibody-positive patients had antibodies to intimin as determined by ELISA, The secreted proteins are expressed in vivo during infection and are considered as pathogenic markers. Antibodies to these proteins may form the basis of a serodiagnostic test for the detection of patients infected with VTEC which carry the locus for the enterocyte effacement pathogenicity island and provide an adjunct test to the established serological tests based on VTEC LPS.
ISSN: 0022-2615



06508323 GENUINE ARTICLE#: RE529 NUMBER OF REFERENCES: 27
TITLE: IDENTIFICATION OF *EAEA"** PROTEIN IN THE OUTER MEMBRANE OF ATTACHING AND EFFACING ESCHERICHIA COLI O45 FROM PIGS
AUTHOR(S): ZHU CR; HAREL J; DUMAS F; FAIRBROTHER JM (Reprint)
CORPORATE SOURCE: UNIV MONTREAL,FAC VET MED,RECH MALAD INFECT PORC GRP,CP 5000/ST HYACINTHE/PQ J2S 7C6/CANADA/ (Reprint); UNIV MONTREAL,FAC VET MED,RECH MALAD INFECT PORC GRP/ST HYACINTHE/PQ J2S 7C6/CANADA/; NATL RES COUNCIL CANADA,BIOTECHNOL RES INST/MONTREAL/PQ H4P 2R2/CANADA/
PUBLICATION: FEMS MICROBIOLOGY LETTERS, 1995, V129, N2-3 (JUN 15), P237-242
ISSN: 0378-1097
LANGUAGE: ENGLISH DOCUMENT TYPE: ARTICLE

ABSTRACT: We have previously reported that the production of attaching and effacing lesions by Escherichia coli 045 isolates from pigs is associated with the *eaeA"** (E. coli attaching and effacing) gene. In the present study, expression of the *EaeA"** protein, the *eaeA"** gene product, among swine 045 E. coli isolates was examined. The majority (20/22) of attaching and effacing positive, *eaeA"**(+) E. coli 045 isolates, but none of ten attaching and effacing negative, *eaeA"**(-) or *eaeA"**(+) isolates, expressed a 97-kDa outer membrane protein as revealed by *sodium"** *dodecyl"** sulfate-polyacrylamide gel *electrophoresis"** (*SDS"**-*PAGE"**) and Western blot analysis. Amino-terminal amino acid sequencing demonstrated a high homology between this 97-kDa protein of swine E. coli 045 and the *EaeA"** protein (intimin) of human enteropathogenic E. coli and enterohemorrhagic E. coli. In addition, a serological relationship between the *EaeA"** proteins of swine 045, rabbit (RDEC-1) and human (E2348/69) attaching and effacing E. coli strains was observed. Our results indicate an association between expression of the *EaeA"** protein and attaching and efficacing activity among 045 E. coli isolates. The data also suggest an antigenic relatedness of the *EaeA"** proteins of swine, rabbit, and human attaching and effacing E. coli.
ISSN: 0378-1097

3/3,AB/4 (Item 3 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)


05603823 GENUINE ARTICLE#: NY149 NUMBER OF REFERENCES: 57
TITLE: PRODUCTION OF ACTIVE, INSECT-SPECIFIC SCORPION NEUROTOXIN IN YEAST
AUTHOR(S): MARTINEAUCLAIRE MF; SOGAARD M; RAMOS C; CESTELE S; BOUGIS PE; SVENSSON B
CORPORATE SOURCE: FAC MED NORD,BIOCHIM LAB,CNRS,URA 1455,BLVD PIERRE DRAMARD/F-13326 MARSEILLE 15//FRANCE/ (Reprint); CARLSBERG LAB,DEPT CHEM/DK-2500 COPENHAGEN//DENMARK/; CARLSBERG LAB,DEPT YEAST GENET/COPENHAGEN//DENMARK/
PUBLICATION: EUROPEAN JOURNAL OF BIOCHEMISTRY, 1994, V223, N2 (JUL 15), P 637-645
ISSN: 0014-2956
LANGUAGE: ENGLISH DOCUMENT TYPE: ARTICLE
ABSTRACT: A cDNA encoding the Androctonus australis Hector insect toxin 1 (AaH IT1) was expressed in yeast leading to secretion of fully biologically active protein. Three different multicopy plasmids were constructed using PCR. Expression was directed by the strong PGK1 promoter of the yeast vector pMA 91. Plasmid pMA 91-AaH IT1 encodes AaH IT1 and its own signal peptide. In the two other constructions, the cDNA encoding the mature part of AaH IT1 is fused to the prepro-signal sequence of the yeast alpha-mating-factor precursor; the pBAL 7-alpha-KREAEA-AaH IT1 includes the cDNA sequence encoding the KR(

*EAEA"**) processing sequence of the alpha-mating factor, and pBAL 7-alpha-KR-AaH IT1 encodes the KR fused directly to the AaH IT1 gene. The yeast alpha-mating-factor signal peptide launched the pro-alpha-mating-factor-AaH IT1 fusion protein into the secretory pathway. The fusion proteins are expected to be cleaved in the Golgi by the KEX2 endopeptidase and the STE13 dipeptidyl aminopeptidase, leading to release of mature AaH IT1. Pulse/chase labelling of transformed yeast protoplasts, followed by *SDS"**/*PAGE"** analysis of proteins immunoprecipitated from either the lysate or the extracellular fluid, showed that AaH IT1 was produced. The highest concentration of recombinant AaH IT1 in the culture medium, as determined using a I-125-AaH IT1 specific radioimmunoassay, was 4 mu g/l (0.5 nM). The recombinant toxin was fully biologically active against cockroaches as assessed by injection and comparison to native AaH IT1. Moreover, it competed with radiolabelled native toxin for its receptor on the voltage-sensitive Na+ channel with a dissociation constant of 0.5 nM.

ISSN: 0014-2956

3/3,AB/5 (Item 1 from file: 348)
DIALOG(R)File 348:European Patents


01047040
ORDER fax of complete patent from Dialog SourceOne. See HELP ORDER 348
Silver halide emulsions with recombinant collagen suitable for photographic application and also the preparation thereof
Fur die photographische Anwendung geeignete Silberhalogenidemulsionen mit rekombinantem Kollagen und deren Herstellung
Emulsions a l'halogenure d'argent avec collagene recombinant approprie pour des applications photographiques

PATENT ASSIGNEE:
Fuji Photo Film B.V., (2597261), P.O. Box 80150, 5000 LJ Tilburg, (NL), (applicant designated states:
AT;BE;CH;CY;DE;DK;ES;FI;FR;GB;GR;IE;IT;LI;LU;MC;NL;PT;SE)
INVENTOR:
van Heerde, George Valentino, Lijndonk 60, 4907 XE Oosterhout, (NL)
van Rijn, Alexis Cornelus, Dommelborch 52, 5247 SG Rosmalen, (NL)
Bouwstra, Jan Bastiaan, Soestdijkseweg-Z 202, 3721 AJ Bilthoven, (NL)
de Wolf, Frederik Anton, Prinses Irenestraat 16, 3981 BR Bunnik, (NL)
Mooibroek, Andreas, Zandweg 8, 6871 TT Renkum, (NL)
Werten, Marc Willem Theodoor, Groen van Prinstererstraat 129, 6702 CR Wageningen, (NL)
Wind, Richele Deodata, Troelstraweg 99, 6702 AH Wageningen, (NL)
van den Bosch, Tanja Jacoba, Laan van Vollenhove 528 bis, 3706 AA Zeist, (NL)
LEGAL REPRESENTATIVE:
de Bruijn, Leendert C. et al (19641), Nederlandsch Octrooibureau P.O. Box 29720, 2502 LS Den Haag, (NL)

PATENT (CC, No, Kind, Date): EP 926543 A1 990630 (Basic)
APPLICATION (CC, No, Date): EP 98204263 981215;
PRIORITY (CC, No, Date): NL 107908 971224
DESIGNATED STATES: DE; FR; GB; NL
INTERNATIONAL PATENT CLASS: G03C-001/005; G03C-001/047; C07K-014/78;

ABSTRACT EP 926543 A1

A tabular silver halide emulsion wherein the tabular grains account for more than 75% of the total grain projected area said emulsion comprising silver halide grains nucleated in the presence of nucleation peptizer and thereafter grown in the presence of growth peptizer, wherein at least one of the peptizers is substantially pure collagen like material prepared by genetic engineering of native collagen encoding nucleic acid, said peptizer having an amino acid sequence comprising more than 4 different amino acids. A process of preparing the AgX emulsion. A process of producing recombinant collagen like polypeptide comprising expression of a collagen like polypeptide encoding nucleic acid sequence by a microorganism to a degree exceeding 0.95 grammes /liter, said recombinant collagen being free of helix structure and preferably the expression occurring in a microorganism other than E. coli or Saccaromyces cerevisiae.

ABSTRACT WORD COUNT: 139

LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

| Available Text | Language | Update | Word Count |
|---|---|---|---|
| CLAIMS A | (English) | 9926 | 1671 |
| SPEC A | (English) | 9926 | 19571 |
| Total word count - document A | | | 21242 |
| Total word count - document B | | | 0 |
| Total word count - documents A + B | | | 21242 |

3/3,AB/6 (Item 2 from file: 348)
DIALOG(R)File 348:European Patents


00988580

CELL SURFACE MOLECULE MEDIATING CELL ADHESION AND SIGNAL TRANSMISSION
ZELLOBERFLACHENMOLEKUL, DAS BEI ZELLADHASION UND SIGNALUBERTRAGUNG EINE ROLLE SPIELT.
MOLECULE DE SURFACE CELLULAIRE INDUISANT L'ADHESION CELLULAIRE ET LA TRANSMISSION DE SIGNAUX
PATENT ASSIGNEE:
Japan Tobacco Inc., (679466), 2-1, Toranomon 2-chome, Minato-ku, Tokyo 105-8422, (JP), (Applicant designated States: all)
INVENTOR:
TAMATANI, Takuya, Jap.Tobacco Inc., 13-2, Fukuura 1-Chome, Kanazawa-ku,

Yokohama-shi, Kanagawa 236-0004, (JP)
TEZUKA, Katsunari, Jap.Tobacco Inc., 13-2, Fukuura 1-chome, Kanazawa-ku,
Yokohama-shi, Kanagawa 236-0004, (JP)
LEGAL REPRESENTATIVE:
VOSSIUS & PARTNER (100314), Siebertstrasse 4, 81675 Munchen, (DE)
PATENT (CC, No, Kind, Date): EP 984023 A1 000308 (Basic)
WO 9838216 980903
APPLICATION (CC, No, Date): EP 98905708 980227; WO 98JP837 980227
PRIORITY (CC, No, Date): JP 9762290 970227; JP 9862217 980226
DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FI; FR; GB; GR; IE; IT; LI; LU; MC; NL; PT; SE
EXTENDED DESIGNATED STATES: AL; LT; LV; MK; RO; SI
INTERNATIONAL PATENT CLASS: C07K-014/705; C07K-016/28; C07K-009/00; C12N-005/10; C12N-015/12; C12P-021/08; A01K-067/027; A61K-038/17; A61K-039/395

ABSTRACT EP 984023 A1
Novel cell surface molecules recognized by monoclonal antibodies against a cell surface molecule of lymphocytic cells that play an important role in autoimmune diseases and allergic diseases have been isolated, identified, and analyzed for their functions. The cell surface molecules are expressed specifically in thymocytes, lymphocytes activated by ConA-stimulation, and peripheral blood lymphocytes, and induce cell adhesion. Antibodies against the cell surface molecules significantly ameliorate pathological conditions of autoimmune diseases and allergic diseases.
ABSTRACT WORD COUNT: 74
NOTE:
Figure number on first page: NONE

LANGUAGE (Publication,Procedural,Application): English; English; Japanese
FULLTEXT AVAILABILITY:

| Available Text | Language | Update | Word Count |
|---|---|---|---|
| CLAIMS A | (English) | 200010 | 1013 |
| SPEC A | (English) | 200010 | 27918 |
| Total word count - document A | | | 28931 |
| Total word count - document B | | | 0 |
| Total word count - documents A + B | | | 28931 |

3/3,AB/7 (Item 3 from file: 348)
DIALOG(R)File 348:European Patents


00985690

Clostridium perfringens vaccine
Clostridium perfringens Impfstoff
Vaccine contre clostridium perfringens

PATENT ASSIGNEE:
Akzo Nobel N.V., (200754), Velperweg 76, 6824 BM Arnhem, (NL), (applicant designated states: AT;BE;CH;CY;DE;DK;ES;FI;FR;GB;GR;IE;IT;LI;LU;MC;NL;PT;SE)
INVENTOR:
Sergers, Ruud Philip Antoon Maria, Groenling 3, 5831 MZ Boxmeer, (NL)
Waterfield, Nicolas Robin, 20 Lucerne Close, Cherry Hinton, Cambridge CB1 4YR, (GB)
Frandsen, Peer Lyng, 56 Borgmester Schneiders Vej, 2840 Holte, (DK)
Wells, Jeremy Mark, The Cottage Old House RD, Balsham, Cambridge CB1 GEF, (GB)
LEGAL REPRESENTATIVE:
Ogilvie-Emanuelson, Claudia Maria et al (80441), Patent Department Pharma N.V. Organon P.O. Box 20, 5340 BH Oss, (NL)
PATENT (CC, No, Kind, Date): EP 892054 A1 990120 (Basic)
APPLICATION (CC, No, Date): EP 98202032 980617;
PRIORITY (CC, No, Date): EP 97201888 970620
DESIGNATED STATES: AT; BE; CH; CY; DE; DK; ES; FI; FR; GB; GR; IE; IT; LI; LU; MC; NL; PT; SE
INTERNATIONAL PATENT CLASS: C12N-015/31; A61K-039/08; C07K-014/33; C12N-001/21;

ABSTRACT EP 892054 A1
The present invention relates to detoxified immunogenic derivatives of Clostridium perfringens (beta)-toxin or an immunogenic fragment thereof that have as a characteristic that they carry a mutation in the (beta)-toxin amino acid sequence, not found in the wild-type (beta)-toxin amino acid sequence. The invention also relates to genes encoding such (beta)-toxins, as well as to expression systems expressing such (beta)-toxins. Moreover, the invention relates to bacterial expression systems expressing a native (beta)-toxin. Finally, the invention relates to vaccines based upon detoxified immunogenic derivatives of Clostridium perfringens (beta)-toxin, and methods for the preparation of such vaccines.
ABSTRACT WORD COUNT: 96

LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

| Available Text | Language | Update | Word Count |
|---|---|---|---|
| CLAIMS A | (English) | 9903 | 583 |
| SPEC A | (English) | 9903 | 7428 |
| Total word count - document A | | | 8011 |
| Total word count - document B | | | 0 |
| Total word count - documents A + B | | | 8011 |

3/3,AB/8 (Item 4 from file: 348)
DIALOG(R)File 348:European Patents

00954338
ORDER fax of complete patent from Dialog SourceOne. See HELP ORDER 348
Non-pathogenic E.Coli mutant strain, process or their preparation and uses
Nicht pathogener mutanter E. coli Stamm, Verfahren zur dessen Herstellung und Verwendung
Souches mutantes non pathogenes d'E.coli, leur procede d'obtention et leurs utilisations
PATENT ASSIGNEE:
INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE (INRA), (222991), 147, rue de l'Universite, F-75341 Paris Cedex 07, (FR), (applicant designated states: BE;DE;ES;FR;IT)
Ecole Nationale Veterinaire de Toulouse (ENVT), (2445840), 23 chemin des Capelles, 31076 Toulouse Cedex, (FR), (applicant designated states: BE;DE;ES;FR;IT)
INVENTOR:
Milon, Alain, 13 avenue du 11 Novembre 1918, 31700 Blagnac, (FR)
De Rycke, Jean, 12 chemin de Larriou, 31820 Pibrac, (FR)
LEGAL REPRESENTATIVE:
Vialle-Presles, Marie Jose et al (75731), Cabinet Ores, 6, Avenue de Messine, 75008 Paris, (FR)
PATENT (CC, No, Kind, Date): EP 864644 A1 980916 (Basic)
APPLICATION (CC, No, Date): EP 98400093 980120;
PRIORITY (CC, No, Date): FR 97532 970120
DESIGNATED STATES: BE; DE; ES; FR; IT
INTERNATIONAL PATENT CLASS: C12N-001/20

ABSTRACT EP 864644 A1 (Translated)
Non-pathogenic Escherichia coli mutants lacking cytopathic phenotype
Non-pathogenic E. coli mutants are produced from a pathogenic strain (cytopathic for HeLa cells and able: (a) to induce formation of actin filaments right through the cells, and (b) to increase the level of vinculin) by mutagenesis and selection for loss of cytopathic capacity.
TRANSLATED ABSTRACT WORD COUNT: 51

ABSTRACT EP 864644 A1
L'invention concerne un procede d'obtention de souches mutantes non-pathogenes d'E. coli, a partir de souches pathogenes capables d'induire sur des cellules epitheliales HeLa un effet cytopathique se manifestant par la formation de cables d'actine polymerisee traversant de part en part lesdites cellules et par une augmentation de la quantite de vinculine.
L'invention concerne egalement les souches mutantes non-pathogenes susceptibles d'etre obtenues par ce procede, et leur utilisation vaccinale.
ABSTRACT WORD COUNT: 69

LANGUAGE (Publication,Procedural,Application): French; French; French
FULLTEXT AVAILABILITY:

| Available Text | Language | Update | Word Count |
|---|---|---|---|
| CLAIMS A | (French) | 9838 | 315 |
| SPEC A | (French) | 9838 | 6654 |
| Total word count - document A | | | 6969 |
| Total word count - document B | | | 0 |
| Total word count - documents A + B | | | 6969 |

3/3,AB/9 (Item 5 from file: 348)
DIALOG(R)File 348:European Patents


00651577
ORDER fax of complete patent from Dialog SourceOne. See HELP ORDER 348
Ligands for FLT3 receptors.
Ligante fur die FLT3 Rezeptoren.
Liants pour les recepteurs FLT3.
PATENT ASSIGNEE:
IMMUNEX CORPORATION, (485033), 51 University Street, Seattle Washington 98101, (US), (applicant designated states: AT;BE;CH;DE;DK;ES;FR;GB;GR;IE;IT;LI;LU;MC;NL;PT;SE)
INVENTOR:
Lyman, Stewart D., 312 N. 4th Street, Seattle, Washington 98115, (US)
Beckmann, M. Patricia, 5454 Ragan Lane, Poulsbo, Washington 98370, (US)
LEGAL REPRESENTATIVE:
Chapman, Paul William et al (73612), Kilburn & Strode, 20 Red Lion Street , London WC1R 4PJ, (GB)
PATENT (CC, No, Kind, Date): EP 627487 A2 941207 (Basic)
EP 627487 A3 960821
APPLICATION (CC, No, Date): EP 94303575 940519;
PRIORITY (CC, No, Date): US 68394 930524; US 106463 930812; US 111758 930825; US 162407 931203; US 209502 940307; US 243545 940511
DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FR; GB; GR; IE; IT; LI; LU; MC; NL; PT; SE
INTERNATIONAL PATENT CLASS: C12N-015/12; C07K-013/00; C12P-021/08; A61K-037/02; C12N-015/87;

ABSTRACT EP 627487 A3
Ligands for flt3 receptors capable of transducing self-renewal signals to regulate the growth, proliferation or differentiation of progenitor cells and stem cells are disclosed. The invention is directed to flt3-L as an isolated protein, the DNA encoding the flt3-L, host cells transfected with cDNAs encoding flt3-L, compositions comprising flt3-L, methods of improving gene transfer to a mammal using flt3-L, and methods of improving transplantations using flt3-L. Flt3-L finds use in treating patients with anemia, AIDS and various cancers.
ABSTRACT WORD COUNT: 91

LANGUAGE (Publication,Procedural,Application): English; English; English

FULLTEXT AVAILABILITY:

| Available Text | Language | Update | Word Count |
|---|---|---|---|
| CLAIMS A | (English) | EPABF2 | 668 |
| SPEC A | (English) | EPABF2 | 15742 |
| Total word count - document A | | | 16410 |
| Total word count - document B | | | 0 |
| Total word count - documents A + B | | | 16410 |

3/3,AB/10 (Item 6 from file: 348)
DIALOG(R)File 348:European Patents


00451134
ORDER fax of complete patent from Dialog SourceOne. See HELP ORDER 348
PREPARATION OF FUSION PROTEINS
HERSTELLUNG VON FUSIONSPROTEINEN
PREPARATION DE PROTEINES DE FUSION
PATENT ASSIGNEE:
HOECHST AKTIENGESELLSCHAFT, (200260), , 65926 Frankfurt am Main, (DE), (applicant designated states: AT;BE;CH;DE;DK;ES;FR;GB;IT;LI;LU;NL;SE)
THE GENERAL HOSPITAL CORPORATION, (370400), 55 Fruit Street, Boston, MA 02114, (US), (applicant designated states: AT;BE;CH;DE;DK;ES;FR;GB;IT;LI;LU;NL;SE)
INVENTOR:
STENGELIN, Siegfried, Kirschgarten 10-12, D-6238 Hofheim am Taunus, (DE)
ULMER, Wolfgang, Unter den Ulmen 6, D-6239 Eppstein/Taunus, (DE)
HABERMANN, Paul, Rossertstrasse 35, D-6239 Eppstein/Taunus, (DE)
UHLMANN, Eugen, Zum Talblick 31, D-6246 Glashutten/Taunus, (DE)
SEED, Brian, 47 A Joy Street, Boston, MA 02114, (US)
LEGAL REPRESENTATIVE:
Aulmich, Gerhard, Dr. et al (58241), Hoechst AG Zentrale Patentabteilung Postfach 80 03 20, W-6230 Frankfurt am Main 80, (DE)
PATENT (CC, No, Kind, Date): EP 489780 A1 920617 (Basic)
EP 489780 A1 920812
EP 489780 B1 981104
WO 9103550 910321
APPLICATION (CC, No, Date): EP 90912715 900828; WO 90US4840 900828.
PRIORITY (CC, No, Date): US 399874 890829
DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FR; GB; IT; LI; LU; NL; SE
INTERNATIONAL PATENT CLASS: C12N-015/62;
NOTE:
No A-document published by EPO
LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

| Available Text | Language | Update | Word Count |
|---|---|---|---|
| CLAIMS B | (English) | 9845 | 325 |
| CLAIMS B | (German) | 9845 | 296 |
| CLAIMS B | (French) | 9845 | 320 |

| | | | |
|---|---|---|---|
| SPEC B | (English) | 9845 | 6966 |
| Total word count - document A | | | 0 |
| Total word count - document B | | | 7907 |
| Total word count - documents A + B | | | 7907 |

3/3,AB/11 (Item 7 from file: 348)
DIALOG(R)File 348:European Patents


00339420
ORDER fax of complete patent from Dialog SourceOne. See HELP ORDER 348
Mutant human angiogenin (angiogenesis factor with superior angiogenin activity) genes therefor and methods of expression.
Menschliches mutantes Angiogenin (Angiogenese-Faktor mit uberlegener Angiogenin-Aktivitat), Gene dafur und Methode zur Expression.
Angiogenine mutante humaine (facteur d'angiogenese avec une activite d'angiogenine superieure), genes pour celle-ci et methode d'expression.
PATENT ASSIGNEE:
THE PRESIDENT AND FELLOWS OF HARVARD COLLEGE, (227952), 17 Quincy Street, Cambridge, MA 02138, (US), (applicant designated states: AT;BE;CH;DE;ES;FR;GB;GR;IT;LI;LU;NL;SE)
INVENTOR:
Harper, Jeffrey W., 1831 Colquitt Street, Houston Texas 77098, (US)
Vallee, Bert L., 56 Browne Street, Brookline Massachusetts 02146, (US)
LEGAL REPRESENTATIVE:
Bizley, Richard Edward et al (28352), HEPWORTH LAWRENCE BRYER & BIZLEY 2nd Floor Gate House South West Gate, Harlow, Essex CM20 1JN, (GB)
PATENT (CC, No, Kind, Date): EP 335243 A2 891004 (Basic)
EP 335243 A3 891123
EP 335243 B1 931103
APPLICATION (CC, No, Date): EP 89105110 890322;
PRIORITY (CC, No, Date): US 173760 880328
DESIGNATED STATES: AT; BE; CH; DE; ES; FR; GB; GR; IT; LI; LU; NL; SE
INTERNATIONAL PATENT CLASS: C12P-021/02; C12N-015/12; A61K-037/02; C12N-001/21;

ABSTRACT EP 335243 A2
Site-specific mutagenesis of a gene for angiogenin producing DNA sequences encoding mutant proteins having increased angiogenic activity are disclosed. Expression vectors containing these sequences are introduced into host cells and direct the production of the mutant angiogenic proteins with markedly increased angiogenic and ribonucleolytic activity. Replacement of a single amino acid, the aspartic acid at or corresponding to position 116 of angiogenin, with another amino acid including asparagine, alanine or histidine, yields mutant proteins with 8 to 15 fold increased ribonucleolytic activity toward tRNA and rRNA and 10 to 100 fold increased angiogenic potency in the chorioallantoic membrane assay. The mutant angiogenin proteins of

this invention are useful therapeutic compositions to promote the development of a hemovascular network in a mammal or to promote wound healing, in particular, healing of torn or traumatized fibrocartilage material.
ABSTRACT WORD COUNT: 139

LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

| Available Text | Language | Update | Word Count |
|---|---|---|---|
| CLAIMS B | (English) | EPBBF1 | 511 |
| CLAIMS B | (German) | EPBBF1 | 551 |
| CLAIMS B | (French) | EPBBF1 | 582 |
| SPEC B | (English) | EPBBF1 | 7061 |
| Total word count - document A | | | 0 |
| Total word count - document B | | | 8705 |
| Total word count - documents A + B | | | 8705 |

3/3,AB/12 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs


0197160 DBA Accession No.: 96-07301
Translational level is a critical factor for the secretion of heterologous proteins in Escherichia coli - enhanced protein secretion; heat-stable enterotoxin-II signal sequence translation initiation region alteration; vector library screening with alkaline phosphatase
AUTHOR: Simmons L C; Yansura D G
CORPORATE AFFILIATE: Genentech
CORPORATE SOURCE: Department of Molecular Biology, Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, CA 94080, USA. email:simmons@gene.com
JOURNAL: Nat.Biotechnol. (14, 5, 629-34) 1996
ISSN: 1087-0156
LANGUAGE: English
ABSTRACT: A method was developed to enhance heterologous protein secretion in Escherichia coli 27C7. Random alteration of the heat-stable enterotoxin-II (STII) signal sequence translational initiation region ( *TIR"** ) resulted in a library of vectors with varying translational strengths. Subsequent library screening using E. coli alkaline phosphatase (EC-3.1.3.1) as a reporter led to the selection of several *TIR"** variants covering a 10-fold range of translational strengths. Transformants were inoculated into 3-5 ml Luria-Bertani medium plus 50 ug/ml carbenicillin and grown at 37 deg with shaking for 4-8 hr. Each culture was then diluted (1:100) into low phosphate media plus carbenicillin and grown for 20 hr at 37 deg with shaking. Whole cell lysates were analyzed by *SDS"**-*PAGE"** . ITR variants enhanced heterologous protein secretion. The heterologous proteins tested required a narrow translational range for optimum high-level secretion